Title Plasma Repressor Element 1-Silencing Transcription Factor Levels Decrease in Patients With Alzheimer's Disease

Mingqing Wei
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine
Jingnian Ni
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine
Jing Shi
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine
Ting Li
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine
Xiaoqing Xu
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine
Chenmeng Li
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine
Bin Qin
Beijing Hospital
Dongsheng Fan
Peking University Third Hospital
Hengge Xie
Chinese PLA General Hospital
Zhong Wang
China Academy of Chinese Medical Sciences
Yongyan Wang
China Academy of Chinese Medical Sciences
Tao Lu
Beijing University of Chinese Medicine
Jinzhou Tian (jztian@hotmail.com)
Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine

---

Research Article

Keywords: Alzheimer's disease, Repressor element 1-silencing transcription, Cognition, Medial temporal-lobe atrophy

Posted Date: February 23rd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-215327/v1

Title

Plasma repressor element 1-silencing transcription factor levels decrease in patients with Alzheimer's disease

Running head

Plasma REST levels decrease in patients with AD

Mingqing Wei, MD[1#], Jingnian Ni, MD[1#], Jing Shi, MD[1], Ting Li, MD[1], Xiaoqing Xu, MD student[1], Chenmeng Li, MD[1], Bin Qin, MD[2], Dongsheng Fan, MD[3], Hengge Xie, MD[4], Zhong Wang, MD[5], Yongyan Wang, MD[5], Tao Lu, PhD[*6], Jinzhou Tian, PhD, MD[1*]

[1]The Neurology Centre, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China

[2]Department of Neurology, Beijing Hospital, Beijing, China

[3]Department of Neurology, Peking University Third Hospital, Beijing, China

[4]Department of Neurology, Chinese PLA General Hospital, Beijing, China;

[5]Institute of Basic Research in Clinical Medicine, China Academy of Chinese Medical Sciences, Beijing China

[6]School of Life Sciences, Beijing University of Chinese Medicine, Beijing, China

[#]These authors were equal contributors.

*Address correspondence:

Jinzhou Tian, the Neurology Centre, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100700, China. E-mail: jztian@hotmail.com

Tao Lu, School of Life Sciences, Beijing University of Chinese Medicine, Beijing 100029, China. Email: 20165102@bucm.edu.cn

ABSTRACT

Background: Repressor element 1-silencing transcription/neuron-restrictive silencer factor (REST) was considered as a new therapeutic target for neurodegenerative disorders like Alzheimer's disease (AD). However, the relationships between AD and REST remain unclear. This study aimed to 1) examine plasma REST levels and REST gene AD patients, and 2) further explore the pathological relationships between REST protein levels and cognition decline in clinic, including medial temporal-lobe atrophy.

Methods: Subjects (n=252, mean age 68.95±8.78 years old) were recruited in Beijing, China, and then divided into normal cognition (NC) group (n=89), amnestic mild cognitive impairment (aMCI) group (n=79) and AD group (n=84) according to diagnostic criteria. All subjects received neuropsychological assessments, laboratory tests and neuroimaging scans (MRI) at baseline. Plasma REST protein levels and distribution of the single-nucleotide polymorphisms (SNPs) of REST were compared across the three groups. Correlation between cognitive function, neuro-image and REST level was calculated using multi-linear-regression analysis . medial temporal-lobe atrophy (need to add this method).

Results: The plasma REST levels in both NC group (430.30±303.43) and aMCI group (414.27±263.39) were significantly higher than AD group (NC vs AD, p=0.034; aMCI vs AD, p=0.033). There was no significant difference between NC and aMCI group (p=0.948). There was no significant difference among three groups on the distribution of the genotype distribution of Rs2227902 and Rs3976529 of REST gene. The REST level was correlated to left medial temporal-lobe atrophy index (r=0.306, p=0.023). After 6-month follow up, the REST level in NC group was positively related to the change scores of mini-mental state examination scale (MMSE)

(r=0.289, p=0.02).

Conclusion: Plasma REST protein declines in AD patients, which also associated with memory impairment and left temporal-lobe atrophy, which may have potential value for clinical diagnosis of AD.

Key words: Alzheimer's disease; Repressor element 1-silencing transcription; Cognition; Medial temporal-lobe atrophy

BACKGROUND

Alzheimer's disease (AD) is a progressive neurodegenerative disease, ranging from preclinical and mild cognitive impairment of symptoms to dementia, accounting for 60-80% of all causes of dementia in population aged over 65[1]. AD-related pathological changes in brains have been verified as accumulation of amyloid-beta protein (Aβ) and neurofibrillary tangles (NFTs) containing hyperphosphorylated tau. However, the pathological mechanisms of AD were still unclear. It was found that the repressor element 1-silencing transcription/neuron-restrictive silencer factor (REST/NRSF) was lost in brain tissues of AD and mild cognitive impairment (MCI) patients. [2]. Similar trend was discovered in plasma neuronal exosomal levels[3,4]. Hence, the REST was considered as a new therapeutic target for neurodegenerative disorders[5]. The neuro-protective function of REST was considered to be correlated with this change: REST down regulated genes that leaded to cell death and AD pathology, and REST protected neurons from oxidative stress and Aβ toxicity[2]. Moreover, REST and its target genes have been implicated in the pathogenesis of a number of different neurodegenerative diseases. Studies have showed that psychomotor speed was also associated with genotype (REST rs3796529) in the longitudinal assessment, and there was a statistically significant association between genotypic variation and memory function at both baseline (NRSF rs2227902 and in longitudinal analysis (REST: rs2227902)[6]. Here we examined REST gene and protein level in plasma to further explore the REST expression The main purpose of this study was to investigate the following questions: (1)are REST levels and REST gene different between normal cognition controls and AD patients; (2) are the REST protein levels correlated to the cognitive decline and medial temporal-lobe atrophy.

METHODS

Participants

Patients, aged from 50 to 85, were recruited in this study from September 2015 to July 2019 in the memory clinic of Dongzhimen Hospital, Beijing University of Chinese Medicine. These Chinese-speaking participants, after giving their informed consent, received a standard neuropsychological assessment, laboratory tests and neuroimaging scans. The neuropsychological assessment include global cognition (Mini-mental state examination, MMSE) and single cognitive domains[7]:(1) episodic memory (Chinese version of Immediately and Delayed story recall, ISR and DSR)[8], (2) visual-spatial skill (Clock Drawing Test, CDT)[9], (3) executive function (Chinese version of the Trail Making Test A,TMT-A)[10], (4) language function (Chinese version of the Boston Name Test, BNT)[11], and ability of daily living scale (ADL)[12], as well as Clinical Dementia Rating scale (CDR) to assess the loss of functioning caused by cognitive impairment [13].

All participants were divided into normal control (NC) group, aMCI group and AD group, according to diagnostic criteria. NC subjects were defined by the Mayo Clinic criteria for healthy controls[14]: (1) Subjects without active neurological or psychiatric disease, (2) no psychotropic medication intake, (3) no medical disorder or its treatment could compromise cognitive function, (4) normal cognitive function evaluated by MMSE score greater than 26; the ADL lower than 16; the CDR score = 0.

Subjects meeting diagnostic criteria for the MCI Working Group of the European Consortium on Alzheimer's Disease were allocated to amnestic type of MCI (aMCI) group[15]: (1) memory complaints reported from the patients or their family members, (2) objective memory impairment, DSR<12.5 adjusted for age, (3) normal general cognitive function as well as no or minimal

impairment in activities of daily living, MMSE score greater than 24 and ADL score lower than 16; CDR = 0.5, and the memory domain=0.5 or 1; (4) absence of dementia judged by an experienced dementia research clinician.

The core clinical criteria of the National Institute on Aging - Alzheimer's Association workgroups was applied to differentiate probable AD[16], and adopted an operational diagnostic standard for AD based on Chinese context[17]: (1) gradual and progressive cognitive function decline over 6 months; (2) significant impairment in episodic memory (DSR<12.5 score) [8] and at least one other cognitive domain (TMT-A>98 second; BNT-30≤22 score; or CDT≤3 score)[10,11]; (3) global cognitive decline evaluated by MMSE adjusted for education (≤22 score for illiterate, ≤23 score for primary school education, ≤24 score for middle school education, ≤26 score for higher education)[18-20]; (4) impaired ADL (ADL ≥16 score)[12]; (5) age-adjusted medial temporal lobe atrophy (MTA-scale) based on coronal MRI of the brain (≥1.0 score aged ≤65, ≥1.5 score aged 66–75, ≥2.0 score aged >75)[21].

Magnetic resonance imaging (MRI) visual rating scale

A standard dementia MRI scan (3.0 Tesla scanner, Siemens, Magnetom verio, Germany) was performed in participants at the department of radiology, Dongzhimen Hospital, Beijing University of Chinese medicine. The image analysis and rating procedures have been described in detail previously[21].

Medial temporal atrophy scale (MTA) was used to assessment of the medial temporal lobe[22], while the global cortical atrophy scale (GCA) was used to assess the global cortical atrophy[23]. The posterior atrophy (PA) rating scale is used for the assessment of posterior atrophy[24]. Medial temporal-lobe atrophy index (MTAi) was used to measure the relative extent of atrophy in medial

temporal lobe in relation to the global cerebral atrophy[25], this method consisted of calculating a ratio with the area of 3 regions, tracing manually on one single coronal MRI slide at the level of the inter peduncular fossa: (1) the medial temporal lobe (MTL) region (A); (2) the parenchyma within the medial temporal region, that includes the hippocampus and the para-hippocampal gyrus—the fimbria taenia and plexus choroideus were excluded(B); and (3) the body of the ipsilateral lateral ventricle (C). From this, we were able to work out the ratio of "Medial Temporal Atrophy index" at both sides as follows: MTAi = (A − B) × 10/C. Two clinicians who were blind to the diagnosis and age of the subjects read the images individually. The result was defined as the average score from two clinicians.

Plasma REST protein and REST gene tests

Before taking blood sample, participants were required to fast for 8 hours. After collection, blood samples were centrifuged at 3000 g for 10 min at 4 °C. Serum was separated and stored in aliquots, and then keep frozen at −80°C until further use. All samples were centrifuged within 2 h of collection. Plasma REST was quantified by human-specific enzyme-linked immunosorbent assay (ELISA) kit for REST (Cusabio, American Research Products, Inc., Waltham, MA). Blood samples were kept in room temperature environment for 30 minutes, and then disposed following the product instruction. DNA was isolated from blood cells using Blood Genomic DNA Extraction Kit (BioTeKe Corporation, Beijing, China). PCR analysis was conducted on 2 genetic locus: Rs2227902 and Rs3976529. The investigator who ran ELISA and polymerase chain reaction (PCR) assays was blind to the group diagnosis.

The protocol was approved by Dongzhimen Hospital, Beijing University of Chinese Medicine Institutional Ethics Committee. The study was undertaken in accordance with the

principles of the Declaration of Helsinki. The patients and responsible caregivers provided written informed consents.

Statistical analysis

Data analysis was performed by SPSS version 21.0 for Windows (IBM, Armonk, NY, USA). Descriptive data were presented as mean value ± standard deviation, while the categorical statistics were given as count and percentage. Group difference of gender, genotype and allele distribution was compared by Chi-square test. Since other descriptive variables including age, education years, neuropsychological test scores, imaging scores and REST level were non-normally distributed, they were compared by non-parametric test. Multi-linear-regression analysis was applied to explore the correlations between REST level (dependent variable) and clinical features including neuropsychological as well as neuro-imaging variables. Statistical significance was set at 0.05. The Receiver operating characteristic (ROC) curves were produced by plotting the sensitivity against the 1-specificity for each score on the plasma REST in discriminating between AD group versus NC group, and between aMCI group versus the NC group.

RESULTS

Participant demographics

A total of 511 subjects were screened in the memory clinic. Among these subjects, 89was diagnosed as NC, 79 as aMCI and 84 as AD patients were included in this study based on the results of neuropsychological assessment and laboratory test. MRI tests were performed in 77 NC, 52 aMCI patients and 55 AD patients. The baseline of characteristics and clinical information are shown in table 1. There was no significant difference in age and gender among three groups, NC

and aMCI group had longer time of education than AD group.

Genotype and allele distribution of REST

Table 2 showed the distribution of the single-nucleotide polymorphisms (SNPs) of REST. There was no significant difference was found in genotype distribution of Rs2227902 and Rs3976529 among three groups. We further assessed the proportion of major or minor alleles and found no statistical difference either among three groups.

Correlation between REST and age

The levels of plasma REST were shown in the Figure 1. There was a significant difference among three groups on the plasma REST level (p=0.048). The REST level in the NC group (430.30±303.43) was significantly higher than AD group (333.08±222.64) (p=0.034). The plasma REST level in the aMCI group (414.27±263.39) was significantly higher than AD group (p=0.033). There was no significant difference between NC and aMCI group (p=0.948).

To explore the correlation between the plasma REST and age, we divided the NC group as three sub-group: age < 65 years, age≥ 65 and age＜75, age ≥ 75years. There was no significant difference among three age groups (p=0.071). When divided all subjects as three sub-group age < 65 years, age≥ 65 and age＜75, age ≥ 75years., there was a significant difference among three sub groups(p=0.013). The REST level in the age < 65 years group (465.14 ±284.59 pg/ml) was significantly higher than the other two age groups(age≥ 65 and age＜ 75371.69±251.42, p=0.021; ≥75years:346.00±260.82, p=0.006).

Correlation between REST and cognition

According to the score of MMSE, we further divided AD patients into three group: mild (20 ≤MMSE<26), moderate(10≤MMSE<20) and severe (MMSE<10), and the results showed there

was no significant difference between different severity of global cognition on plasma REST .

The correlation between REST level and clinical features (including neuropsychological assessments and MRI visual rating scale)was analyzed, which indicated that left MTAi (r=0.306, p=0.023) and ISR (r=0.526, p=0.040) were both positively related to REST level, while no significant correlation was discovered in other variables.

Correlation between REST and cognitive changes after 6 months

64 NC subjects were followed up for 6 months from the first visit, and took the same neuropsychological test during the visit. The correlation between the results of neuropsychological tests and the baseline level of REST level was calculated. The baseline level of REST was related to the MMSE change score from 6 month to baseline (r=0.289, p=0.02), there was no significant correlation between the scores of neuropsychological tests (including DSR, ISR, CDT, TMT-A, CDR-SB) and the baseline level of REST.

Sensitivity and specificity of plasma REST for discriminate AD

ROC analysis was performed to calculate the cutoff score and diagnostic value of plasma REST level for discriminating AD from NC (Figure 2). The area under curve (AUC) was 0.593 (P =0.043) (95% CI: 0.509–0.678). When the cutoff values were 477pg/ml for plasma REST, the sensitivity (38.2%) and specificity (78.6%) was obtained for distinguishing AD from NC. The AUC for distinguishing NC subjects from aMCI patients was 0.497 for plasma REST (95% CI: 0.409–0.585). The AUC for distinguishing aMCI patients from AD patients was 0.597 for REST (95% CI: 0.509-0.685).

DISCUSSION

In this cross-sectional study, it was indicated that the level of REST protein in plasma

decreased in the cognitive dysfunction group, while the level of REST expression was extremely lower in the AD group, and the level of REST was correlated to the memory function and medial temporal-lobe atrophy index.

In recent years, REST, as a neuroprotective factor, has been paid more and more attention. Lu et al. had firstly showed REST was increasingly expressed in human cortical and hippocampus neurons during ageing. The study showed that REST levels were reduced by only 40% in MCI but 1.5 - fold more at 60% in nucleus of cortical and hippocampus neurons in AD[2] . And our results were consistent with the previous study. In our study, AD patients showed lower plasma REST level, compared to the NC group and aMCI group. The plasma REST level was tested using ELISA, and the trend of plasma REST was consistent with of REST in the nucleus. The results of our study supported the idea that REST might be a neuro-protective factor, and the loss of REST may leads to further development of the pathological changes of AD.

Studies has found that the level of REST protein in the nucleus of AD patients is related to the maintenance of cognitive function. In brains of AD patients, Lu et al. found that almost total absence of REST in neuronal nuclei of the prefrontal cortical neurons, and nuclear REST levels were significantly correlated with measures of episodic, semantic, and working memory [2], but it hasn't been reported whether the level of plasma REST is correlated with cognition. Our study found that the level of REST protein in plasma is positively correlated with the memory function which was measured by instant memory recall scale, but there was no correlation between the global cognition and REST. Mean while, the baseline plasma REST was correlated with the change scores of MMSE after 6-month follow-up, and this indicated that the baseline REST may predict changes in global cognition.

In addition, our study also found a positive correlation between plasma REST levels and MTAi-left score, which was consisted to previous study[26]. suggesting that REST may serve as an independent risk marker for AD.

The idea that REST gene polymorphism has a protective effect on patients with MCI or AD remains controversial. Some studies have sequenced the genes of rs3796529 and rs2227902 in European and American populations, and shown that MCI patients carrying allele T at rs3796529 have more larger hippocampus volume and slower atrophy rate. However, it has not been shown whether the rs3796529 variant could protect the hippocampus of people with normal cognitive[27]. It has been reported that the rs3796529 variant has a neuro-protective effect in healthy people and MCI patients [28], but another study showed that the rs3796529 variant has nothing to do with hippocampus volume. On the contrary, the minor T allele of the rs2227902 variant seems to be related to a decrease in the volume of the right hippocampus [29]. The conclusions of these studies were inconsistent. In this study, there was no significant difference among three groups about the REST gene polymorphisms, and there is no correlation with the hippocampus volume. Further studies and discussions are still needed in this area.

In current clinical practice, the plasma REST showed relative lower sensitivity for distinguishing AD patients from NC subjects and MCI patients. But another study showed the relative higher the sensitivity for distinguishing NC subjects from AD patients for plasma REST (confidence interval [CI]: 100%–100%)[30]. The possible reason for this difference was the test method we used was not sensitive.

LIMITATION

This study also exists some limitation. Firstly, the sample is relatively small, the above

conclusion may need to be further verified in a larger sample. Secondly, whether the peripheral plasma REST protein level represents the level in neurons is still controversial. However, the above research evidence still provides a certain method and basis for carrying out REST testing in clinical research.

CONCLUSION

In conclusion, plasma REST protein declines in AD patients and may be associated with memory function and left temporal-lobe atrophy, the plasma REST may have potential value for clinical diagnosis of AD. However, due to a relatively small sample, this conclusion need to be further verified in a larger population.

DECLARATIONS

- **Ethics approval and consent to participate:** This study was approved by Dongzhimen Hospital, Beijing University of Chinese Medicine Institutional Ethics Committee. The study was undertaken in accordance with the principles of the Declaration of Helsinki. The patients and responsible caregivers provided written informed consents.
- **Consent for publication:** Not applicable.
- **Availability of data and material:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.
- **Competing interests:** There are no competing interests.
- **Funding:** This study was supported by the project from the fundamental research funds for the central universities(No. 2019-JYB-TD-007), Program for Changjiang Scholars and Innovative Research Team in University(No:IRT0810).

- **Authors' contributions:** Jinzhou Tian, Tao Lu and Yongyan Wang designed the study. Mingqing Wei and Jingnian Ni wrote the manuscript. Mingqing Wei, Jingnian Ni, Jing Shi, Ting Li, Xxiaoqing Xu, Chenmeng Li, Bin Qin, Dongsheng Fan, Hengge Xie performed the neuropsychological assessment, Zhong Wang did the analysis. Jinzhou Tian and Tao Lu were principal investigators for this study and finalized the manuscript. All authors approved the final manuscript.

- **Acknowledgements**: Not applicable.

REFERENCES

(1) What is Alzheimer's | Alzheimer's Association," https://www.alz.org/alzheimers-dementia/what-isalzheimers.

(2) Lu T, Aron L, Zullo J, et al. REST and stress resistance in ageing and Alzheimer's disease. Nature. 2014;507(7493):448-454..

(3) Abner EL, Jicha GA, Shaw LM, Trojanowski JQ, Goetzl EJ. Plasma neuronal exosomal levels of Alzheimer's disease biomarkers in normal aging. Ann Clin Transl Neurol. 2016;3(5):399-403.

(4) Goetzl EJ, Boxer A, Schwartz JB, et al. Low neural exosomal levels of cellular survival factors in Alzheimer's disease. Ann Clin Transl Neurol. 2015;2(7):769-773.

(5) Hattori N. REST as a new therapeutic target for neurodegenerative disorders. Mov Disord. 2014;29(7):869.

(6) Warburton A, Miyajima F, Shazadi K, Crossley J, Johnson MR, Marson AG, Baker GA, Quinn JP, Sills GJ. NRSF and BDNF polymorphisms as biomarkers of cognitive dysfunction in adults with newly diagnosed epilepsy. Epilepsy Behav. 2016;54:117-27.

(7) Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-198.

(8) Shi J, Wei M, Tian J, et al. The Chinese version of story recall: a useful screening tool for mild cognitive impairment and Alzheimer's disease in the elderly. BMC Psychiatry. 2014;14:71.

(9) Wolf-Klein GP, Silverstone FA, Levy AP, Brod MS. Screening for Alzheimer's disease by clock drawing. J Am Geriatr Soc. 1989;37(8):730-734.

(10) Wei M, Shi J, Li T, et al. Diagnostic Accuracy of the Chinese Version of the Trail-Making Test for Screening Cognitive Impairment. J Am Geriatr Soc. 2018;66(1):92-99.

(11) Gguo Q, Hong Z, Shi W, et al. Boston Naming Test in Chinese Elderly, Patient with Mild Cognitive Impairment and Alzheimer's Dementia. Chinese Mental Health Journal, 2006,20(2):81-84.

(12) Ni J, Shi J, Wei M, et al. Screening mild cognitive impairment by delayed story recall and instrumental activities of daily living. Int J Geriatr Psychiatry. 2015;30(8):888-890.

(13) Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical scale for the staging of

dementia. Br J Psychiatry. 1982;140:566-572.

(14) Harris ME, Ivnik RJ, Smith GE. Mayo's Older Americans Normative Studies: expanded AVLT Recognition Trial norms for ages 57 to 98. J Clin Exp Neuropsychol. 2002;24(2):214-220.

(15) Portet F, Ousset PJ, Visser PJ, et al. (2006), Mild cognitive impairment (MCI) in medical practice: a critical review of the concept and new diagnostic procedure. Report of the MCI Working Group of the European Consortium on Alzheimer's Disease. Journal of neurology, neurosurgery, and psychiatry 77:714-718.

(16) Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):270-279.

(17) Tian J, Liang X, Shi J, et al. Diagnosis and Treatment of Alzheimer’s Disease. 2010, Beijing, China: People’s Health Press.

(18) Tsoi KK, Chan JY, Hirai HW, Wong SY, Kwok TC. Cognitive Tests to Detect Dementia: A Systematic Review and Meta-analysis. JAMA Intern Med. 2015;175(9):1450-1458.

(19) O'Bryant SE, Humphreys JD, Smith GE, et al. Detecting dementia with the mini-mental state examination in highly educated individuals. Arch Neurol. 2008;65(7):963-967.

(20) Zhang Z, Hong X, Li H.The Mini-mental State Examination in the Chinese Residents population aged 55 years and over in the urban and rural areas in Beijing. Chinese Journal of Neurology,1999,32(3):149.

(21) Wei M, Shi J, Ni J, et al. A new age-related cutoff of medial temporal atrophy scale on MRI improving the diagnostic accuracy of neurodegeneration due to Alzheimer's disease in a Chinese population. BMC Geriatr. 2019;19(1):59.

(22) Scheltens P, Leys D, Barkhof F, et al. Atrophy of medial temporal lobes on MRI in "probable" Alzheimer's disease and normal ageing: diagnostic value and neuropsychological correlates. J Neurol Neurosurg Psychiatry. 1992;55(10):967-972.

(23) Ferreira D, Cavallin L, Larsson EM, et al. Practical cut-offs for visual rating scales of medial temporal, frontal and posterior atrophy in Alzheimer's disease and mild cognitive impairment. J Intern Med. 2015;278(3):277-290.

(24) Koedam EL, Lehmann M, van der Flier WM, et al. Visual assessment of posterior atrophy development of a MRI rating scale. Eur Radiol. 2011;21(12):2618-2625.

(25) Menéndez-González M, López-Muñiz A, Vega JA, Salas-Pacheco JM, Arias-Carrión O. MTA index: a simple 2D-method for assessing atrophy of the medial temporal lobe using clinically available neuroimaging. Front Aging Neurosci. 2014;6:23.

(26) Ashton NJ, Hye A, Leckey CA, et al. Plasma REST: a novel candidate biomarker of Alzheimer's disease is modified by psychological intervention in an at-risk population. Transl Psychiatry. 2017;7(6):e1148.

(27) Nho K, Kim S, Risacher SL, et al. Protective variant for hippocampal atrophy identified by whole exome sequencing. Ann Neurol. 2015;77(3):547-552.

(28) Dols-Icardo O, Vilaplana E, Sampedro F, et al. Effect of REST on brain metabolism in the Alzheimer disease continuum. Ann Neurol. 2015;78(4):661-662.

(29) Mozzi A, Guerini FR, Forni D, et al. REST, a master regulator of neurogenesis, evolved under strong positive selection in humans and in non human primates. Sci Rep. 2017;7(1):9530.

(30) Winston CN, Goetzl EJ, Akers JC, et al. Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. Alzheimers Dement (Amst). 2016;3:63-72.

Table 1 Baseline characteristics of study participants

Items	NC (n=89)	aMCI (n=79)	AD (n=84)
Age (y)	68.60±9.28	69.16±8.55	69.13.±8.54
Male (%)	63（54.78%）	45（47.37%）	43（43.88%）
Education（y）	13.07±7.25	11.04±3.17*	10.83±4.48*△
Neuropsychological tests			
n	89	79	84
MMSE	27.96±1.25	26.71±1.71*	17.00±5.77**△△
ISR	25.58±10.61	12.10±6.40**	2.34±2.75**△△
DSR	23.26±11.73	6.03±4.20**	0.60±1.67**△△
CDT	3.95±0.22	3.78±0.50	2.41±1.33**△△
TMT-A	67.74±27.23	71.26±29.01	122.07±33.11**△△
CDR-SB	0.78±0.98	1.42±1.20*	6.31±3.12**△△
ADL	14.08±0.28	14.28±0.45*	23.16±6.19*△
MRI visual rating scale			
n	77	52	55
MTA-right	0.62±0.76	0.98± 0.92*	1.76± 1.02**△△
MTA-left	0.79± 0.86	0.96 ±0.91	1.89 ±1.05**△△
GCA	0.74 ±0.75	1.11 ±0.91*	0.97±0.86
PA	0.62 ± 0.66	0.94 ±0.72**	1.12± 0.66**△△
MTAi-right	1.97 ± 1.48	2.71± 1.46**	3.47 ±1.71**△
MTAi-left	1.71± 1.49	2.35± 1.37*	3.42 ±1.58

Notes: NC, Normal control; aMCI, amnestic Mild cognitive impairmentt; AD, Alzheimer's disease; MMSE, Mini Mental State Examination; ISR, Instant Story Recall; DSR, Delayed Story Recall; CDT, Clock Drawing Test; TMT-A, Trail Making Test A; CDR-SB, Clinical Dementia Rating Sum of Boxes; ADL, Activities of Daily Living. * $p < 0.05$ when AD or aMCI group VS NC; ** $p < 0.01$ when AD or aMCI group VS NC;△ $p < 0.05$ when AD VS aMCI group; △ $p < 0.05$ when AD VS aMCI group; MTA, medial temporal lobe atrophy scale; GCA, global cortical atrophy scale; PA, posterior atrophy; MTAi, Medial temporal-lobe atrophy index (Medial temporal lobe region - the parenchyma within the medial temporal region) × 10/ the body of the

ipsilateral lateral ventricle.

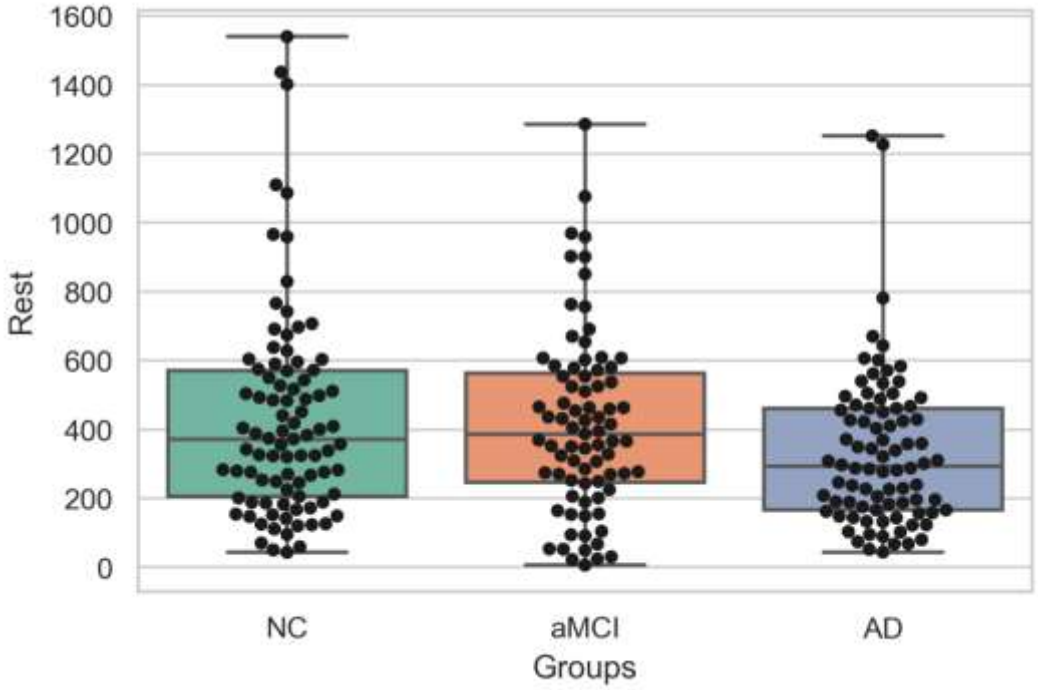
Table 2 Genotype and allele distribution of REST single-nucleotide polymorphisms of study participants

·		Allele	Genotype n（%）			p	Alleles n（%）		p
	N	M/m	MM	Mm	mm		M	m	
Rs2227902		G/T							
NC	89		81(92.0%)	5(5.7%)	2(2.3%)	0.772	167 (94.9%)	9(5.1%)	0.849
aMCI	79		71(89.9%)	7(8.9%)	1(1.3%)		149(94.3%)	9(5.7%)	
AD	84		74(88.1%)	9(10.7%)	1(1.2%)		157(93.5%)	11(6.5%)	
Rs3976529		C/T							
NC	89		31(34.8%)	42(47.2%)	16(18.0%)	0.779	104(58.4%)	74(41.6%)	0.954
aMCI	79		26(33.3%)	42(53.8%)	12(12.8%)		94(58.8%)	66(41.2%)	
AD	84		26(32.5%)	44(55.0%)	10(12.5%)		96(60.0%)	64(40.0%)	

Notes: REST, RE-1 silencing transcription factor; NC, Normal control; aMCI, amnestic Mild cognitive impairment; AD, Alzheimer’s disease; M, Major allele; m, Minor allele

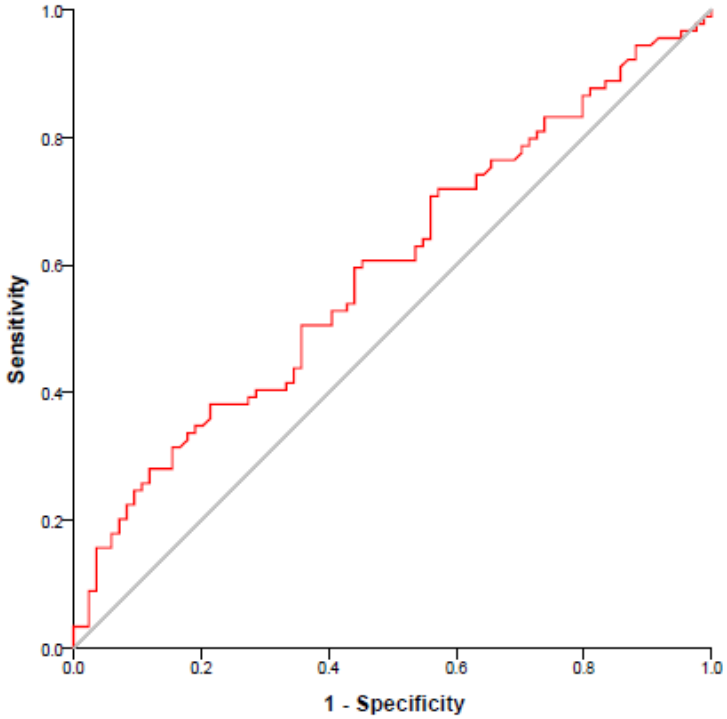
Figure legends

Figure 1 Comparison plasma REST level between three groups subjects.



Notes: NC, Normal control; aMCI, amnestic Mild cognitive impairment; AD, Alzheimer's disease; REST, RE-1 silencing transcription factor ; *p<0.05 NC or aMCI vs AD group.

Figure 2 ROC curve analyses assess diagnostic accuracy for plasma REST for NC subjects versus AD patients



Notes: NC, Normal control; AD, Alzheimer's disease; REST, RE-1 silencing transcription factor ; ROC curve, Receiver operating characteristic curve

Figures

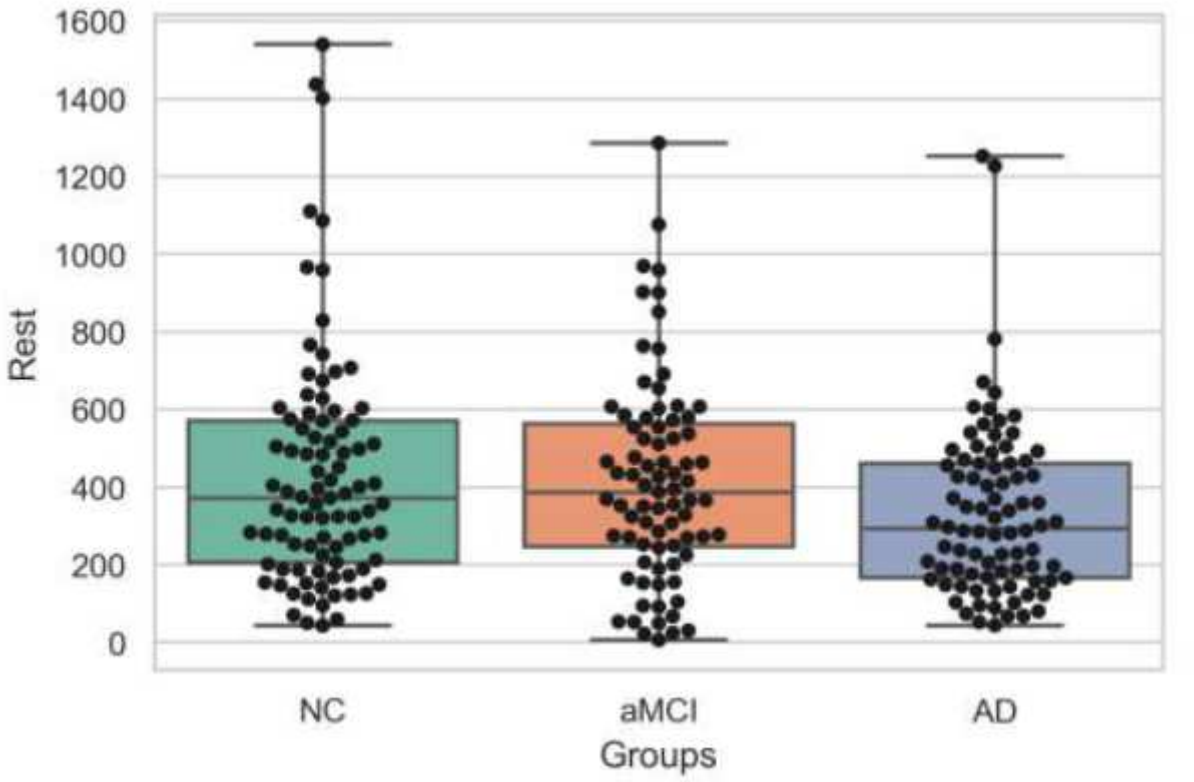


Figure 1

Comparison plasma REST level between three groups subjects. Notes: NC, Normal control; aMCI, amnestic Mild cognitive impairment; AD, Alzheimer's disease; REST, RE-1 silencing transcription factor ; *p<0.05 NC or aMCI vs AD group.

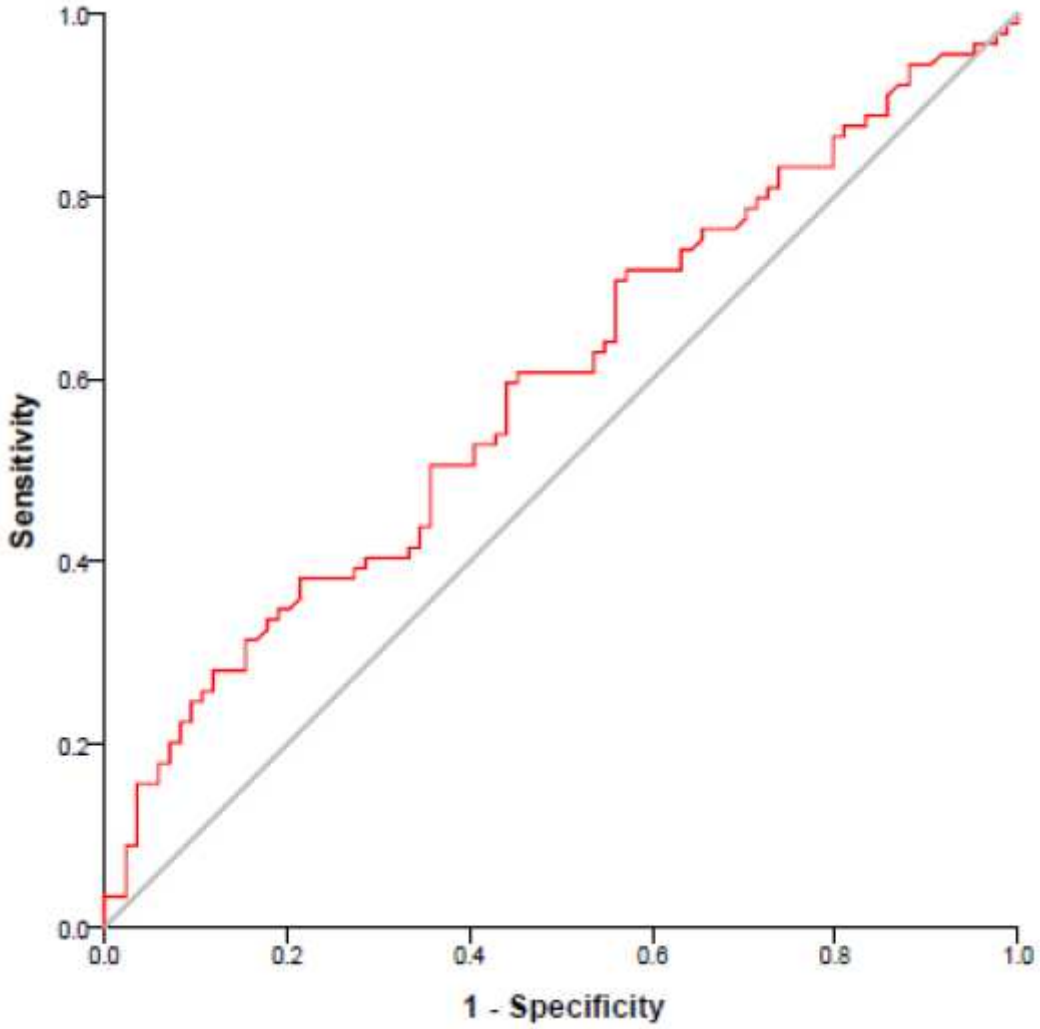


Figure 2

ROC curve analyses assess diagnostic accuracy for plasma REST for NC subjects versus AD patients
Notes: NC, Normal control; AD, Alzheimer's disease; REST, RE-1 silencing transcription factor ; ROC curve, Receiver operating characteristic curve