Cerebrospinal Fluid α-Synuclein Predicts Neurodegeneration and Clinical Progression in Prodromal Alzheimer's Disease

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Research

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Abstract

Background

Accumulating reports suggest that α-synuclein is involved in Alzheimer disease (AD) pathogenesis. Cerebrospinal fluid (CSF) α-synuclein could be a potential biomarker of AD. We sought to test whether CSF α-synuclein is associated with other AD biomarkers and could predict neurodegeneration and clinical progression in prodromal AD.

Methods

Associations were investigated between CSF α-synuclein and other AD biomarkers at baseline in prodromal AD stage Chinese elders. The predictive values of CSF α-synuclein in longitudinal change in clinical outcomes and conversion risk of prodromal AD stage subjects were assessed using linear mixed effects models and multivariate Cox proportional hazard models, respectively, in Alzheimer's disease Neuroimaging Initiative (ADNI) database.

Results

Among individuals in prodromal AD stage, we detected that CSF α-synuclein levels correlated with AD-specific biomarkers CSF total tau and phosphorylated tau levels in 651 Chinese Han participants (training set). These positive correlations were replicated in ADNI database (validation set). Using a longitudinal cohort from ADNI, CSF α-synuclein concentrations increased with disease severity. CSF α-synuclein had high diagnostic accuracy for AD based on the “ATN” system (A+T+) vs controls (A-T-) (area under the receiver operating characteristic curve, 0.84). Moreover, CSF α-synuclein predicted longitudinal hippocampus atrophy and conversion from MCI to AD dementia.

Conclusions

CSF α-synuclein is associated with CSF tau levels and could predict neurodegeneration and clinical progression in prodromal AD. This finding indicates CSF α-synuclein is a potentially useful, early biomarker for AD.

Background

Alzheimer's disease (AD) is the leading cause of dementia in the elderly and is defined clinically by a gradual decline in memory and other cognitive functions. However, less than half of the patients with dementia received a formal diagnosis in Europe and the USA.[1] Pathological change is extrapolated to 20 years before clinical symptom onset of AD. Biomarker research made it possible to identify people at high risk of developing dementia in the general population, even at the preclinical stage.[2, 3] According to the newly published “ATN” scheme, various biomarkers were divided into three binary components: (i) biomarkers of β-amyloid (Aβ) plaques or associated pathophysiologic processes labeled “A”; (ii) biomarkers of aggregated pathologic tau or associated pathophysiologic processes labeled “T”; (iii)
Biomarkers of neurodegeneration or neuronal injury labeled “N”.[4] Besides the biomarkers mentioned above, additional novel biomarkers that reflect other disease mechanisms may provide insight into the different mechanisms implicated in AD pathogenesis and assist in identifying novel targets for therapies in the future. This was echoed by the 2018 NIA-AA research framework that “ATN” can be expanded to incorporate other proteinopathies which were also involved in AD pathogenesis or frequently co-occurred with AD pathologic changes.[5-7] This provided a multidimensional approach to diagnosing dementia and better clinical stratification of patients for therapeutic trials.[8, 9]

α-synuclein, best known for its role in Parkinson's disease (PD) and dementia with Lewy bodies (DLB), has been reported to be implicated in AD pathogenesis.[10] Patients with AD and concomitant α-synuclein pathology typically had a more rapid rate of cognitive decline than subjects with AD alone.[11, 12] α-synuclein was generally considered as pre-synaptic protein, which is also been found in human cerebrospinal fluid (CSF).[13, 14] Many studies found CSF α-synuclein was identified as an appropriate biomarker for PD and other synuclein-associated diseases,[15-17] especially for the diagnostic differentiation of different neurodegenerative diseases.[18, 19] However, studies on the potential role of CSF α-synuclein as a biomarker for the presymptomatic phase of AD remains unclear.

In this study, we explored the associations between CSF α-synuclein and other AD biomarkers in nondemented Chinese elderly adults. We also tested whether CSF α-synuclein is altered in patients with AD and different AD pathophysiological profiles based on “ATN” classifications and whether it is associated with other AD biomarkers, cognitive decline and imaging evidence of neurodegeneration in the ADNI database. The value of CSF α-synuclein as a predictor of disease progression and neurodegeneration at the presymptomatic stages of AD was also investigated.

**Methods**

**Study participants**

Six hundred and fifty-one subjects in prodromal stage of AD who were northern Han Chinese in origin were derived from Chinese Alzheimer’s Biomarker and Life style (CABLE) study. CABLE is a large cohort study mainly focusing on Alzheimer’s risk factors and biomarkers in Chinese elderly adults. The samples in CABLE study were recruited at Qingdao Municipal Hospital, consisting of cognitively normal (CN) elders as well as individuals with MCI. All participants were Han Chinese in origin aged 50 to 90 years. Controls had MiniMental State Examination (MMSE) scores of 24 or higher, where lower scores indicate more impairment and higher scores less impairment (range, 0-30), and a Clinical Dementia Rating (CDR) score of 0, where lower scores indicate less impairment and higher scores more impairment (range, 0-3). Patients with MCI had MMSE scores of 24 or higher, objective memory loss tested by delayed recall of the Wechsler Memory Scale (WMS) logical memory II (>1 SD below the normal mean), a CDR score of 0.5, preserved activities of daily living, and absence of dementia. The exclusion criteria were: (1) central nervous system infection, head trauma, epilepsy, multiple sclerosis or other major neurological disorders; (2) major psychological disorders (e.g., depression); (3) severe systemic diseases (e.g., malignant tumors)
that may affect CSF or blood levels of AD biomarkers including Aβ and tau; (4) family history of genetic disease. All participants underwent clinical and neuropsychological assessments, biochemical testing, as well as blood and CSF sample collection. Demographic information, AD risk factor profile and medical history were also collected by a comprehensive questionnaire and an electronic medical record system.

Data were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu), an independent replication cohort. The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, as well as clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. For up-to-date information, see www.adni-info.org.

Our ADNI cohort included all CN controls, MCI patients and AD patients with available baseline CSF α-synuclein samples. Inclusion/exclusion criteria are described at http://www.adni-info.org. In our study, we stratified the MCI group into stable MCI (sMCI) with no progression to AD dementia during at least 2-year follow-up, and progressive MCI (pMCI) with progression to AD dementia during at least 2-year follow-up. Therefore, we included the following 4 groups: CN controls, sMCI group, pMCI group and AD group. As to “ATN” binary (i.e., positive or negative) categories: amyloid positive (A+) and negative (A-) were separated by a cutoff value of 192 pg/ml for CSF Aβ level. Tau pathology positive (T+) and negative (T-) were separated by a cutoff value of 23 pg/ml for CSF phosphorylated tau (p-tau) level.

The CABLE study was approved by the Institutional Ethics Committees of Qingdao Municipal Hospital. Written informed consent was obtained from all study participants directly or from their caregivers. The ADNI study was approved by the Institutional Review Board at each of the participating centers, and all participants provided written informed consent.

**CSF Measurements**

CSF was taken by lumbar punctures through the L3/L4 interspace and gently mixed to avoid gradient effects. The samples were promptly centrifuged at 2000 g for 10 min to eliminate cells and other insoluble materials, aliquoted in 1 ml portions, snap frozen at −80°C until use. CSF was sampled between 08:00 and 09:00 in the morning in order to take into account a possible circadian rhythm effect. The CSF samples were stored at −80°C until further analysis of Aβ and tau.

In CABLE study, CSF Aβ42, total tau (t-tau), p-tau and CSF total α-synuclein concentrations were measured separately using an enzyme-linked immunosorbent assay (ELISA) kit (Fujirebio, Ghent, Belgium). All the ELISA measurements were performed according to the manufacturers’ instructions. The samples and standards were measured in duplicate, and the means of the duplicates were used for the statistical analyses.
In ADNI database, CSF Aβ42, t-tau and p-tau were measured at the ADNI biomarker core (University of Pennsylvania) using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX, USA) with the INNOBIA AlzBio3 kit (Fujirebio, Ghent, Belgium). Levels of CSF total α-synuclein concentrations in the ADNI cohort were measured by Luminex MicroPlex Microspheres (Luminex Corp, Austin, TX). A biotinylated goat antihuman α-syn antibody (R&D systems, Minneapolis, MN, USA) was used as the detection antibody. The α-synuclein Luminex assay demonstrated low day-to-day and plate-to-plate signal variability. The accuracy of the assay was further determined by the recovery of spiked α-synuclein protein, which was close to 93 %.

**Neuroimaging**

Structural MRI was performed only for the ADNI subjects using a Siemens Trio 3.0T scanner or Vision 1.5T scanner (GE, Siemens and Philips). Free-surfer software package version 4.3 and 5.1 image processing framework were used to process regional volume estimates for the 1.5 and 3.0T MRI images, respectively. ROIs included the hippocampus and ventricles.

**Statistical analyses**

We tested associations between CSF α-synuclein and demographic factors using the Mann-Whitney test and the Spearman rank correlation test. We tested the associations of CSF α-synuclein with CSF Aβ42, t-tau, and p-tau levels using linear regression adjusted for age, gender, educational level, diagnosis and APOE ε4 genotype (with CSF α-synuclein as predictor). In the ADNI database, associations between CSF α-synuclein concentrations and the diagnostic groups were tested in an analysis of covariance model adjusted for age, gender, educational level and APOE ε4 genotype. Logistic regression analysis was used to assess the impact of different CSF analytes on the risk of conversion to AD. The receiver-operator curves and the area under the curves were derived from the predictive probabilities of the logistic regression models. We tested the associations of CSF α-synuclein concentrations with longitudinal cognition and brain structure using linear mixed-effects models. These models had random intercepts and slopes for time and an unstructured covariance matrix for the random effects and included the interaction between (continuous) time and CSF α-synuclein as predictor with adjustment for confounders. All tests were 2-sided. Statistical significance was set at P < 0.05. All regression analyses were corrected for age, gender, educational level, diagnosis, and APOE ε4 genotype. The following variables were natural log-transformed to ensure normality: CSF α-synuclein, p-tau, t-tau, Aβ and hippocampus volume. All statistical analyses were performed using a software program (R, version 3.4.0; The R Foundation).

**Results**

**Characteristics of Participants in CABLE Study**
We included 651 subjects in prodromal stage of AD from the CABLE study consisting of 457 CN controls (238 women, 60.54 ± 10.46 years) and 194 MCI patients (109 women, 63.6 ± 9.72 years) (Table 1). CN individuals were younger and more educated. CSF p-tau and t-tau levels were higher in MCI patients than CN individuals.

| Demographics for the Study Population in CABLE a |
|-----------------------------------------------|
| CN (n=457) | MCI (n=194) | P Value |
| AGE, mean (sd), y | 60.93 (10.55) | 65.44 (10.01) | <0.001 |
| Female, No. (%) | 269 (58.9) | 109 (56.2) | 0.59 |
| APOE ε4 genotype carriers, No. (%) | 69 (15.1) | 35 (18.0) | 0.41 |
| Educate, mean (SD), y | 10.38 (6.12) | 8.56 (4.23) | <0.001 |
| CSF α-synuclein, mean (SD), ng/L | 1466.73 (813.99) | 1501.19 (914.13) | 0.61 |
| CSF PTAU, mean (SD), ng/L | 38.11 (9.69) | 40.04 (12.42) | 0.03 |
| CSF TAU, mean (SD), ng/L | 173.3 (77.96) | 191.02 (122.57) | 0.03 |
| CSF ABETA42, mean (SD), ng/L | 160.01 (91.51) | 162.10 (105.53) | 0.81 |

Abbreviations: Aβ, β-amyloid; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; t-tau, total tau.

a P values are from the Kruskal-Wallis test or Fisher exact test.

**CSF α-synuclein and Established AD Biomarkers in CABLE Study**

In CABLE study, we examined the concentrations of CSF α-synuclein and other established AD biomarkers (CSF Aβ, p-tau and t-tau) and tested their relationships (Table 2). We found that high CSF α-synuclein level is associated with high CSF t-tau (β = 0.56, P < 0.001) and p-tau (β = 0.35, P < 0.001) among nondemented subjects. However, there were no associations between CSF α-synuclein and CSF Aβ level at baseline. We also tested these relationships in subgroups. The results were the same in the CN group (CSF t-tau: β = 0.38, P < 0.001, CSF p-tau: β = 0.27, P < 0.001) and MCI group (CSF t-tau: β = 0.67, P < 0.001, CSF p-tau: β = 0.4, P < 0.001).
| Biomarker | All Participants | CN       | MCI       |
|-----------|-----------------|----------|----------|
|           | β Coefficient   | P Value  | β Coefficient | P Value | β Coefficient | P Value |
| CSF t-tau | 0.56            | <0.001   | 0.38      | <0.001   | 0.67         | <0.001  |
| CSF p-tau | 0.35            | <0.001   | 0.27      | <0.001   | 0.40         | <0.001  |
| CSF Aβ42  | -0.02           | 0.97     | -0.01     | 0.82     | -0.07        | 0.69    |

Abbreviations: Aβ, β-amyloid; CABLE, Chinese Alzheimer's Biomarker and Lifestyle; CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; p-tau, phosphorylated tau; t-tau, total tau.

a Data are β coefficients (with P values) from linear regression models for correlations between CSF α-synuclein and other biomarkers, adjusted for age, gender, educational level and APOE ε4 genotype. Models were tested in the whole cohort and in individual diagnostic groups.

**Characteristics of Participants in ADNI**

Three hundred and eighty-two subjects from the ADNI database were included (Table 3). This cohort consisted of 109 CN controls (54 women, 75.63 ± 5.22 years), 117 sMCI patients (37 women, 74.34±7.60 years), 66 pMCI patients (25 women, 74.21± 7.58 years) and 90 AD patients (39 women, 74.89±7.72 years). According to the new “ATN” scheme, 258 A+ (220 A+T+) patients and 124 A- (96 A-T-) controls were included. As expected, the AD group had the highest frequency of the ε4 allele within APOE gene (69.23%) and the CN controls group had the lowest frequency (23.85%). There was no significant difference in educational level (P = 0.16) and age (P = 0.53) among these four groups. Furthermore, AD patients had lower MMSE scores compare with MCI patients and CN controls (P < 0.01).
Table 3
Demographics for the Study Population in ADNI

|                      | CN (n = 109) | sMCI (n = 117) | pMCI (n=66) | AD (n =90) |
|----------------------|--------------|----------------|-------------|------------|
| Age, mean (SD), y    | 75.63(5.22)  | 74.34(7.60)    | 74.21(7.58) | 74.89(7.72)|
| Female, No. (%)      | 54(49.54)    | 37(31.62)      | 25(36.76)   | 39(44.32)  |
| APOE ε4 genotype carriers, No. (%) | 26(23.85) | 55(47.00)      | 42(61.76)   | 63(69.23)  |
| CSF α-synuclein, mean (SD), ng/L | 0.46(0.17) | 0.54(0.22)     | 0.56(0.20)  | 0.61(0.24) |
| MMSE score, mean (SD) | 29.07(1.05) | 27.15(1.64)    | 26.58(1.77) | 23.39(1.80)|
| CSF Aβ42, mean (SD), ng/L | 208.70(52.36) | 174.69(55.28) | 148.75(41.52) | 143.99(38.31) |
| CSF t-tau, mean (SD), ng/L | 69.08(29.85) | 97.31(64.77)   | 112.00(41.52) | 122.83(57.09) |
| CSF p-tau, mean (SD), ng/L | 25.04(13.93) | 32.76(18.31)   | 39.50(17.18) | 41.48(19.73) |
| Hippocampus volume, mm3 | 6648.16(766.59)| 5964.07(986.76)| 5522.46(1044.15) | 5217.39(1043.40) |

Abbreviations: Aβ, β-amyloid; AD, Alzheimer disease dementia; CN, cognitively normal; CSF, cerebrospinal fluid; sMCI, stable mild cognitive impairment; pMCI, progressive mild cognitive impairment; MMSE, Mini-Mental State Examination; p-tau, phosphorylated tau; t-tau, total tau.

CSF α-synuclein and Established AD Biomarkers in ADNI

In ADNI database, we found that high CSF α-synuclein levels were associated with high CSF t-tau (β = 0.27, P < 0.001) and p-tau (β = 0.36, P < 0.001) in the whole cohort. However, there was no association between CSF α-synuclein and CSF Aβ level at baseline. The same results were obtained in the MCI group (CSF t-tau: β = 0.29, P = < 0.001, CSF p-tau: β = 0.33, P < 0.001) and CN controls (CSF t-tau: β = 0.2, P = < 0.001, CSF p-tau: β = 0.32, P < 0.001).

CSF α-synuclein in Different Diagnostic Groups in ADNI

With the advance of the disease stage, the level of CSF α-synuclein showed a rising trend. The CSF α-synuclein concentration was significantly higher in the AD and pMCI groups compared with CN controls (P < 0.0001 and P < 0.001 respectively). Higher CSF α-synuclein levels were also detected in the AD and pMCI groups compared with the sMCI group (P = 0.02 and P = 0.04, respectively) (Figure 1A). We continued to compare CSF α-synuclein concentration among A- controls, A+ controls, A- patients with
MCI, A+ patients with MCI and A+ patients with AD dementia (Figure 1B). The A+ AD group had higher CSF α-synuclein levels than those of A- controls (P < 0.001), A+ controls (P < 0.001), and A- MCI group (P < 0.001). The A+ MCI had higher CSF α-synuclein levels than those of A- controls (P < 0.01), A+ controls (P < 0.01), and A- MCI group (P = 0.02). We further compared the CSF α-synuclein level between the A+T+ group with the A-T- group, which showed differences with more significant statistical power (P < 0.0001) (Figure 1C).

We performed receiver-operating curves based on the logistic regression models adjusted for age at baseline, gender, educational level and APOE ε4 genotype to assess the predictive value of CSF α-synuclein and its combination with other established AD biomarkers in the risk of conversion to AD. The area under the curve (AUC) of the base model containing CSF α-synuclein, age at baseline, gender, educational level and APOE ε4 genotype was 0.76 in predicting the onset of AD among CN controls, and AUC was increased by the inclusion of CSF tau/Aβ ratio (AUC = 0.88) (Figure e-1). Consistent with expectation, the base model showed similar predicting value in the onset of pMCI among CN controls (Figure e-2). In the A- group, this base model showed pretty good predictive value in the risk of conversion to A+ status (AUC = 0.77). This value became greater when combined with CSF t-tau (AUC = 0.88) and p-tau (AUC = 0.92) (Figure e-3). Furthermore, when the participants were grouped according to both Aβ deposition and pathology, the base model showed the best performance (AUC = 0.84) (Figure e-4).

**CSF α-synuclein and Longitudinal Clinical Outcomes Change and Progression in ADNI**

Next, the linear mixed-effects models were utilized to test the associations between baseline CSF α-synuclein concentration and subsequent disease progression adjusted for age, gender, educational level, diagnosis, and APOE ε4 genotype. A Significant association of baseline CSF α-synuclein concentration with hippocampus volume was identified (β = -0.008, P = 0.001 longitudinally) (Table 4, Figure 2A).
Table 4
Modelling the association of CSF biomarkers on AD biomarkers and clinical outcomes in ADNI a

|                                | All Participants | MCI | CN  |
|--------------------------------|------------------|-----|-----|
|                                | β Coefficient    | P Value | β Coefficient | P Value | β Coefficient | P Value |
| **Cross-sectional (MR)**       |                  |        |                |        |                |         |
| CSF t-tau                      | 0.27             | < 0.001 | 0.29           | < 0.001 | 0.20           | < 0.001 |
| CSF p-tau                      | 0.36             | < 0.001 | 0.33           | < 0.001 | 0.32           | < 0.001 |
| CSF Aβ42                       | -0.03            | 0.33   | -0.04          | 0.32   | 0.006          | 0.86    |
| **Longitudinal (MELM)**        | β Coefficient    | P Value | β Coefficient | P Value | β Coefficient | P Value |
| Hippocampus                    | -0.008           | 0.001  | -0.007         | 0.04   | -0.003         | 0.17    |
| Ventricles                     | 0.006            | 0.13   | 0.005          | 0.36   | 0.003          | 0.43    |
| **Cox (Hazard ratio)**         | Statistic        | P value |
| MCI conversion to AD Dementia  | 1.53(1.15-2.0)   | 0.004  |

a All models are adjusted for age, gender, educational level, APOE ε4 genotype and intracranial volume (for MRI only). Models were tested in the whole cohort and in individual diagnostic groups.

Abbreviations: CN, cognitively normal; CSF, cerebrospinal fluid; MCI, mild cognitive impairment; p-tau, phosphorylated tau; t-tau, total tau; Cox, Cox proportional hazard model; MELM, mixed effects linear model; MR, multiple regression.

Figure 2B presents the results of a Kaplan-Meier analysis. The cox proportional hazards model was developed to estimate the predictive value of CSF α-synuclein in the conversion risk from MCI to incident AD dementia, controlling for baseline age, gender and years of education. MCI individuals with high CSF α-synuclein levels would satisfy the diagnostic criteria for AD at a comparatively earlier interval (HR 2.79, 95% CI 1.14 to 6.9, P = 0.03) (Table 4).

**Discussion**

The main findings of this study were that CSF α-synuclein concentration (1) associated with CSF t-tau and p-tau levels among nondemented elderly adults, (2) was elevated in AD dementia group and in Aβ/tau-positive group compared with control groups, (3) predicted longitudinal hippocampus atrophy and conversion from MCI to AD dementia. Taken together, these findings suggest that CSF α-synuclein is a
very early and potentially presymptomatic biomarker for AD. This biomarker may be helpful in AD diagnosis, predicting disease progression and staging severity of AD even in its preclinical stage. Our study also provided clues to how α-synuclein participated in the pathogenetic process in AD and provided evidence for drug development.

“Pure” AD is characterized by the presence of both diffused neuritic plaques and intracellular neurofibrillary tangles, which lacks abnormal α-synuclein inclusions or neuritis. However, more than 50 % AD patients exhibit abundant brain accumulation of α-synuclein-positive Lewy bodies, particularly in the amygdala.[10, 20] The presence of α-synuclein does not appear to be innocuous, as these patients demonstrate an accelerated cognitive decline than subjects with AD alone.[12, 21] Previous studies indicated that α-synuclein could be secreted into the surrounding media in the brain and then to the CSF. Studying CSF could provide clues to the mechanism of α-synuclein metabolism in brain.

Consistent with most studies, our study showed that CSF α-synuclein was higher in AD group compared with CN controls and MCI group. A possible hypothesis is that the higher level of α-synuclein in the AD group is caused by the higher expression of α-synuclein in the brains of AD patients. The higher expression of α-synuclein could induce a decrease in selected synaptic vesicle proteins and alteration of the protein composition of synaptic vesicles, thus causing neuronal damage in AD which, in turn, increases the release of α-synuclein from damaged cells into the CSF.[22] As CSF α-synuclein levels were significantly higher in patients with AD than in those with synucleinopathies (CSF α-synuclein was lower than controls), indicating α-synuclein might serve as a biomarker for differential dementia diagnosis. Logistic regression analysis was used to assess the impact of CSF analytes on risk for progression to AD. The AUC (reflect predictive probabilities of the logistic regression models) of the model including CSF α-synuclein, age at baseline, gender, educational level and APOE ε4 genotype was great in predicting progression from CN to pMCI or AD. Recently, the NIA-AA committee recommended a different definition of AD by pathophysiology which is independent from clinical symptoms. They proposed that as long as biomarker evidence of Aβ and tau pathology was present simultaneously, the term “Alzheimer’s disease” would be applied. And the CSF α-synuclein model had high diagnostic accuracy for patients with the diagnosis of AD based on the “ATN” system (A+T+) vs controls (A-T-) (AUC = 0.84, which is comparable to other established CSF biomarkers).

Many lines of evidence suggested that pathological α-synuclein, Aβ and tau may have synergistic adverse effects to promote their mutual aggregation, thereby amplifying neuronal damage [22-28]. Notably, α-synuclein inclusions are commonly observed in patients with familial Alzheimer’s disease or Down’s syndrome in which Aβ peptides are highly expressed. In both diseases, α-synuclein affects biological pathways and promotes the formation of Aβ aggregates. α-synuclein was supposed to be implicated in synaptic vesicle formation, axonal transport as well as dopamine synthesis and metabolism.[29] In the normal condition, the synaptic membrane is integrated and the α-synuclein is completely released to the cytosol. However, in the event of neuronal damage and synaptic membrane defect, both aggregated Aβ and α-synuclein might attach to synaptic membrane and accumulate in lipid rafts. Synaptic membrane-bound α-synuclein could not only induce cytosolic α-synuclein to aggregate as
intracellular LBs but also interact with membrane-associated Aβ_{40} and Aβ_{42} peptides.[30] This could explain the low level of CSF α-synuclein in initial stages of AD to a certain extent. Moreover, an in vitro experiment demonstrated that interaction with Aβ_{1–42} is sufficient to induce the intracellular accumulation of α-synuclein, whereas interaction with Aβ_{1–40} is not.[31] In our study, although we did not detect any association between CSF α-synuclein and CSF Aβ levels at baseline, the strong interaction between them in brain could not be denied. The reason may be that this mutual effect happens in the initial stages of the mixed pathology, however, it may take years or decades for intracellular α-synuclein to be available in extracellular space and eventually detectable in the CSF. We only studied CSF total α-synuclein level rather than the oligomeric or phosphorylated forms. Future study focusing on oligomeric or phosphorylated forms of α-synuclein may provide additional information.

Moreover, α-synuclein was also being observed in progressive supranuclear palsy[32] and frontotemporal dementia.[33] Many studies proposed that α-synuclein and tau interact to promote each other's fibrillation and toxicity.[23] However, as α-synuclein could spontaneously polymerize into amyloidogenic fibrils, tau requires cofactors such as glycosaminoglycans or nucleic acids.[34] The α-synuclein polymers act as amyloidogenic “seeds” or as amyloidogenic chaperones that induce the formation of tau fibrillar inclusions even in the absence of α-synuclein coexpression.[23, 24, 35] Besides, Tau promotes α-synuclein to polymerize into fibrils. Low concentrations of α-synuclein don't fibrillize without tau, however, in the presence of tau, most α-synuclein assembles into fibrils. Much attention has been paid to the relationship between CSF α-synuclein and tau. Most studies demonstrated that CSF α-synuclein was positively associated with CSF t-tau and p-tau.[22, 26] Our study also indicated positive associations of CSF α-synuclein with CSF t-tau and p-tau levels in CABLE study. We noted that the mean values for CSF α-synuclein and CSF Aβ levels between controls in the 2 Chinese cohorts using similar assays are different. This could partly be explained by differences in pre-analytical protocols, analytical procedures, assays quality together with discrepancies in absolute levels between assay formats[36]. Replication studies with larger sample sizes are warranted to confirm the present findings.

Importantly, we found that the CSF α-synuclein levels might correlate with AD severity and progression. Our finding was consistent with a recent study indicating that increased α-synuclein displayed a stronger association with cognitive impairment than soluble Aβ and tau levels.[37] It has been widely recognized that α-synuclein is a synaptic marker. α-synuclein is highly expressed in the pre-synaptic terminals[38, 39] and it plays a role in the regulation of neurotransmitter release, synaptic function and plasticity. It could trigger synaptotoxicity not only by directly damaging the synaptic membrane, but also by damaging mitochondria, lysosomes, or by disrupting microtubules. This then leads to dendritic and spine alterations, axonal dystrophy, and eventually neuronal loss.[40] Along with the synaptic damage, α-synuclein is released into the cerebrospinal fluid. Therefore, it is reasonable to assume that CSF α-synuclein level correlates with cognitive decline in AD, since synaptic damage is supposed to be a strong predictor of cognitive decline.[41]

Conclusions
CSF α-synuclein was associated with CSF t-tau and p-tau levels among nondemented elderly adults. In ADNI database, CSF α-synuclein concentrations were increased with the severity of the disease. CSF α-synuclein predicted longitudinal hippocampus atrophy and conversion from MCI to AD dementia. The current findings suggest CSF α-synuclein as a very early and potentially presymptomatic biomarker for AD, a prognostic marker in the clinic, and an outcome measure in clinical trials.

List Of Abbreviations

ADNI: Alzheimer's Disease Neuroimaging Initiative

Aβ: biomarkers of β-amyloid

AD: Alzheimer disease

CSF: Cerebrospinal fluid

CABLE: Chinese Alzheimer's Biomarker and Life style

CN: cognitively normal

CDR: Clinical Dementia Rating

DLB: dementia with Lewy bodies

MMSE: MiniMental State Examination

MRI: magnetic resonance imaging

MCI: mild cognitive impairment

PET: positron emission tomography

PD: Parkinson's disease

Declaration

Ethics approval and consent to participate

The CABLE study was approved by the Institutional Ethics Committees of Qingdao Municipal Hospital. ADNI study were approved by the institutional review boards of all participating centres (https://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf), and written informed consent was obtained from all participants or their authorised representatives. Ethics approval was obtained from the institutional review boards of each institution involved: Oregon Health and Science University; University of Southern California; University of California—San Diego; University of Michigan; Mayo Clinic, Rochester; Baylor College of Medicine; Columbia University Medical Center;
Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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**Authors’ contributions**

Dr Jin-Tai Yu had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Jin-Tai Yu, Jie-Qiong Li.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Jie-Qiong Li.

Critical revision of the manuscript for important intellectual content: Jin-Tai Yu, John Suckling, Hui-Fu Wang, Qiang Dong, Lan Tan, Qiang Liu, Yan-Jiang Wang, Chuan-Tao Zuo, Can Zhang

Statistical analysis: Jie-Qiong Li.

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**Figures**

![Figure 1](image)

**Figure 1**

CSF α-synuclein, diagnosis, and AD pathophysiology in ADNI Cerebrospinal fluid α-synuclein concentrations in the diagnostic groups are shown as scatterplots. The colors of the scatterplots are grouped by different diagnostic groups. The three horizontal black lines in each boxplot indicate the median and interquartile range. The whiskers extend to the minimum and maximum CSF α-synuclein data points. (A). CSF α-synuclein concentration in the diagnostic groups. (B). CSF α-synuclein concentration in the diagnostic groups stratified by Aβ pathology. (C). CSF α-synuclein concentration in the AD pathophysiology (tau and amyloid-β) positive and negative. Abbreviations: A-, Aβ negative; A+, Aβ positive; T-, tau negative; T+, tau positive.
Figure 2

Associations between CSF α-synuclein and longitudinal clinical outcomes change in ADNI Data from (A) linear mixed-effects models and (B) cox proportional hazards models adjusted for age, gender, educational level and APOE ε4 genotype. All participants classified into binomial groups according to their baseline CSF α-synuclein concentration.