

Received: 2021.05.04

Accepted: 2021.07.11

Available online: 2021.07.16

Published: 2021.07.27

# The Association Between Plasma $\alpha$ -Synuclein ( $\alpha$ -syn) Protein, Urinary Alzheimer-Associated Neuronal Thread Protein (AD7c-NTP), and Apolipoprotein Epsilon 4 (ApoE $\epsilon$ 4) Alleles and Cognitive Decline in 60 Patients with Alzheimer's Disease Compared with 28 Age-Matched Normal Individuals

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Statistical Analysis C  
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**Source of support:** The study was supported by a grant from the National Key R&D Program of China (Project No. 2016YFC1306300), the National Natural Science Foundation of China (Grant No. 81974220) and the Central health research project (grant No. 2020ZD10)

**Background:** Accumulating evidence has shown that  $\alpha$ -synuclein ( $\alpha$ -syn) pathology is involved in the pathophysiology of Alzheimer's disease (AD). This study aimed to investigate the association between the levels of plasma  $\alpha$ -syn protein, urinary Alzheimer-associated neuronal thread protein (AD7c-NTP), apolipoprotein epsilon 4 (ApoE  $\epsilon$ 4) alleles and cognitive decline in 60 AD patients compared with 28 age-matched normal controls (NCs) at a single center.





**Material/Methods:** All participants underwent  $\alpha$ -syn, apolipoprotein E (ApoE), AD7c-NTP, cholesterol (CHO), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides (TGs) analyses, neuropsychological scale assessments and neuroimaging analysis. Moreover, urine and peripheral blood samples were collected from all participants. The levels of plasma  $\alpha$ -syn and AD7c-NTP were assayed using an enzyme-linked immunosorbent assay (ELISA) kit. Other test results were obtained from China-Japan Friendship Hospital.

**Results:** We found that plasma  $\alpha$ -syn levels were significantly different between AD patients and NCs ( $p=0.045$ ).  $\alpha$ -Syn levels were also associated with AD7c-NTP ( $r=0.231$ ,  $p=0.03$ ) but not ApoE  $\epsilon$ 4 ( $Z=-0.147$ ,  $p=0.883$ ) levels. Neither  $\alpha$ -syn [CHO ( $p=0.432$ ), HDL ( $p=0.484$ ), LDL ( $p=0.733$ ) or TGs ( $p=0.253$ )] nor AD7c-NTP [CHO ( $p=0.867$ ), HDL ( $p=0.13$ ), LDL ( $p=0.57$ ) or TGs ( $p=0.678$ )] had a relationship with lipids.

**Conclusions:** This study showed that the levels of plasma  $\alpha$ -syn protein and urinary AD7c-NTP were significantly increased in AD patients compared with NCs, but not with ApoE alleles or serum lipid levels.

**Keywords:** **AD7c-NTP Protein, Human • alpha-Synuclein • Alzheimer Disease • Apolipoprotein E4**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/932998>

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## Background

Alzheimer's disease (AD) is an irreversible neurodegenerative disease in the central nervous system with clinical features of memory loss and cognitive decline [1]. Amyloid- $\beta$  (A $\beta$ ) and tau are involved in the pathophysiological processes underlying AD [2]. AD is the most common type of dementia, accounting for 3/5-4/5 of all cases [3]. AD is a thorny problem for both society and families. Unfortunately, a recent study showed that 99.6% of clinical trials ended in failure during 2000 and 2012 [4]. Once dementia is apparent, it may be too late to initiate the treatment for AD, which cascades toward inevitable outcomes [5]. Thus, it is important to identify AD biomarkers and define the relationships among them.

Recent studies have shown that approximately 1/3 of AD patients have cerebral  $\alpha$ -synuclein ( $\alpha$ -syn) depositions [6]. Wang's study showed that serum  $\alpha$ -syn levels were different between AD and Parkinson's disease dementia through enzyme-linked immunosorbent assay (ELISA) [7]. A study injected  $\alpha$ -syn preformed fibrils into transgenic mice with 5 familial Alzheimer's disease mice (5xFAD). A $\beta$  deposits accelerated  $\alpha$ -syn protein in the brain of 5xFAD [8]. The accumulation and spreading of tau was lower in the brains of mice without endogenous  $\alpha$ -syn than in those of blank control mice [9]. A $\beta$ 42 and  $\alpha$ -syn can promote each other's oligomerization [10,11]. In particular,  $\alpha$ -syn stimulates the oligomerization of A $\beta$ 42, which leads to its precipitation and the formation of hybrid ring-like structures [12]. Thus, accumulating evidence has shown that  $\alpha$ -syn pathology is involved in the pathophysiology of AD.

Another study showed that urinary Alzheimer-associated neuronal thread protein (AD7c-NTP) co-localizes with tau-immunoreactive neurofibrillary tangles [13]. Furthermore, overexpression of AD7c-NTP is related to neurite sprouting and cell death, which is closely associated with the pathophysiological mechanisms underlying AD [14]. The urine AD7c-NTP level is elevated in AD, which has also been identified as a biomarker for AD patients [15]. Similarly, the apolipoprotein E (ApoE) allele is also associated with tau-immunoreactive neurofibrillary tangles and A $\beta$  deposition [16]. Three common alleles of ApoE –  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4 – are defined by 2 single-nucleotide polymorphisms (rs429358 and rs7412) that reside in the coding region of exon 4. They overlap with a well-defined CpG island (CGI). A study showed that normal elderly individuals with ApoE  $\epsilon$ 4 homozygotes ( $\epsilon$ 4/ $\epsilon$ 4) have a very high risk of developing clinical AD [17]. It is known that the risk of AD is increased 3- to 4-fold in individuals with only one  $\epsilon$ 4 allele compared to patients without  $\epsilon$ 4 alleles [18]. Apolipoproteins and  $\alpha$ -syn, with their amphipathic helices, insert into lipid membranes and influence their curvature [19]. Wojciech Paslawski's study [20] showed that in patients with Parkinson's disease,  $\alpha$ -syn interacts with ApoE in human cerebrospinal fluid (CSF). Thus,  $\alpha$ -syn may have a relationship with ApoE.

All 3 biomarkers participate in the pathophysiological processes of A $\beta$  and tau. The study also showed that they were higher in AD patients [7,15,17]. However, the relationship among them remains unclear. All these biomarkers also have a relationship with lipids [21,22]. ApoE is a lipid carrier in not only the peripheral nervous system but also the central nervous system [23]. A study showed that  $\alpha$ -syn plays a role in the regulation of synaptic vesicle fusion and is dynamic at the membrane interface, which suggests  $\alpha$ -syn-lipid interactions [24]. A study of 2180 participants showed that after adjusting for age and sex, there were significant differences in urinary AD7c-NTP levels between blood lipids [21]. We aimed to explore their relationships and provide evidence that they can reflect the risk of AD development. Then, we explored whether cholesterol (CHO), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglycerides (TGs) were associated with these biomarkers. Combining these biomarkers and examining their relationships may have clinically meaningful benefits.

Therefore, this study aimed to investigate the association between the levels of plasma  $\alpha$ -syn protein, urinary AD7c-NTP, and apolipoprotein epsilon 4 (ApoE  $\epsilon$ 4) alleles and cognitive decline in 60 patients with AD compared with 28 age-matched normal individuals at a single center.

## Material and Methods

### Study Population

The study was reviewed and approved by our ethics committee and institutions (Ethics ID: 2017SY51). All subjects signed an informed consent form at the beginning of the study. The purpose of our study and the principle of voluntary participation were explained to each subject. AD patients must be accompanied by their fiduciaries. The study population included 60 AD patients and 28 normal controls (NCs). All of them were selected from the China-Japan Friendship Hospital from 2018 March to 2021 January.

### Clinical Diagnostic Criteria

The subjects were recruited by 3 experienced doctors (Dr. Peng, Dr. Wang, and Dr. Jia) through the Department of Neurology of China-Japan Friendship Hospital. Sixty subjects were diagnosed with AD based on the diagnostic criteria of the National Institute on Aging-Alzheimer's Association (NIA-AA) [25,26], with the Standardized Mini-Mental State Examination (MMSE)  $\leq$ 23, Montreal Cognitive Assessment (MoCA)  $\leq$ 23, Clinical Dementia Rating (CDR)  $>$ 0.5, Geriatric Depression Scale (GDS)  $<$ 11, and Hachinski Ischemia Scale (HIS)  $<$ 4. In addition, all subjects underwent neuroimaging analysis (magnetic resonance imaging; MRI) to rule out other brain diseases leading to cognitive

decline. Moreover, we conducted ApoE genotyping, and eligible subjects were invited for the baseline visit. At the same time, we recruited 28 healthy subjects of similar sex and age as those in the AD group for the control group.

### Inclusion Criteria

The inclusion criteria were as follows: (1) men or women; (2) age between 45 and 95 years old; (3) right-handed; (4) native Chinese individuals; (5) AD diagnosis established by NIA-AA criteria or NCs; and (6) AD patients accompanied by their fiduciaries who voluntarily signed the informed consent form.

### Exclusion Criteria

The exclusion criteria were as follows: (1) severe AD and cognitive impairment caused by cerebral blood disease; (2) a history of stroke, brain tumor, or other nervous system diseases and serious heart, liver, lung, kidney and other systemic diseases; (3) moderate to severe depression and other mental diseases; and (4) occurrence of other neurological diseases or the presence of cardiac pacemakers, coronary stents, dental implants and other metal implants, cochlear implants and other ferromagnetic materials. Participants who failed to follow the study protocol were also excluded.

### Urine AD7C-NTP Assay

Each subject collected 10-ml, first, clean-catch, and midstream urine samples in the morning. Then, the urine samples were stored at 4°C. Levels of AD7c-NTP were assayed using an ELISA kit (catalog no.2400416, Anqun Biological Technology Co. Ltd., Shenzhen, China). All experiments were performed according to the manufacturer's instructions [27]. Then, each plate was washed with phosphate-buffered saline (PBS). Next, urine samples were incubated with biotinylated rabbit anti-AD7c-NTP antibody at 37°C for 1h. Then, each plate was washed with PBS. Horseradish peroxidase-labeled avidin was added to each plate and incubated at 37°C for 30 min. Each plate was washed with PBS. Then, 50 mL of chromogenic reagents A and B was added to each plate and incubated at 37°C for 15min. Finally, 50 mL of sulfuric acid was added to stop the reaction. The reaction product was measured at an optical density (OD) value of 450 nm with a multimode reader (Multiskan MK3, Thermo Scientific, Waltham, Massachusetts, United States).

### Plasma $\alpha$ -syn Protein Assay

Peripheral blood (4 ml) was collected from each subject in the morning. The plasma was centrifuged and separated by centrifugation (CR312, Jouan, Evreux, Eure, France). Then, the plasma samples were stored at -80°C. Plasma concentrations of  $\alpha$ -syn protein were measured by ELISA kits with reagents for human

detection (catalog no. ab260052, Abcam, Cambridgeshire, United Kingdom). The details of the experiments were as described above. The reaction product was measured at an OD value of 450 nm with a multimode reader (Multiskan MK3, Thermo Scientific, Waltham, Massachusetts, USA).

### Statistical Analysis

We used SPSS Version 20 (Armonk, United States) to perform the statistical analyses in our study. The data were first defined to determine whether the distribution of the data was normal. All the data were non-normally distributed in our study. Medians and interquartile ranges (IQRs) were used for non-Gaussian distributed variables at baseline. Two-group comparisons, such as the analysis of differences in baseline characteristics between the AD and NC groups, were analyzed by the 2 independent-samples Mann-Whitney U test (unpaired). When the expected count was less than 5, the Fisher chi-square test was used instead of the chi-square test. Spearman correlation analyses were used. The association of clinical and biological characteristics was evaluated through linear and multivariable regression analyses. A two-tailed  $P < 0.05$  was considered a significant difference in all statistical analyses.

## Results

### Participant Demographic Data

The study population included 60 AD patients with an average age of  $69.77 \pm 10.18$  (female: 40, male: 20) and 28 NCs with an average age of  $70.00 \pm 8.07$  (female: 18, male: 10). There were no significant differences in age, sex, education, smoking, alcohol use, blood pressure or lipids (including CHO, HDL, LDL and TGs) between the 2 groups (shown in **Table 1**). Neuropsychological assessment scales (including the MMSE, MoCA and Activities of daily living [ADL]), plasma  $\alpha$ -syn level and AD7c-NTP levels were different between the 2 groups.

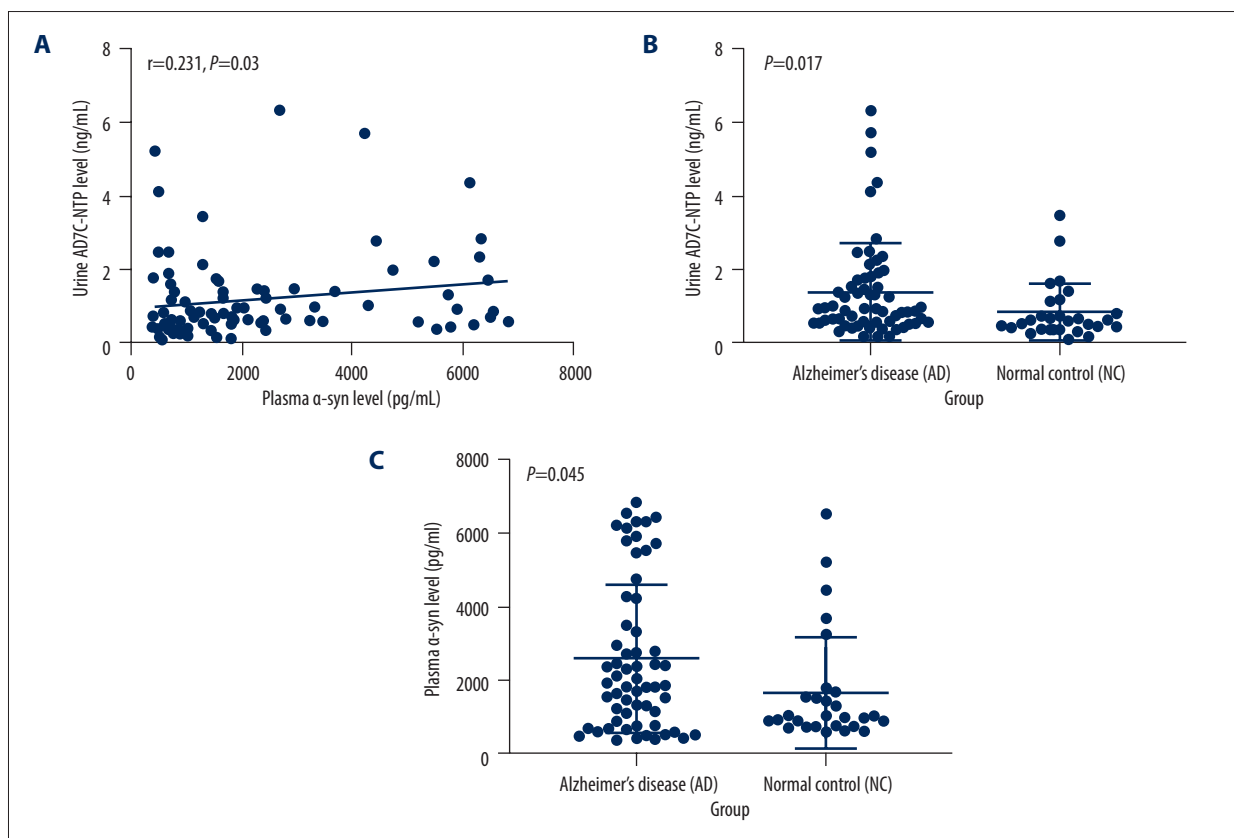
### Relationship Between Plasma $\alpha$ -syn and Urinary AD7c-NTP Levels

The concentration of plasma  $\alpha$ -syn in the AD group was higher than that in the NC group ( $Z = -2.007$ ,  $p = 0.045$ ). Spearman correlation analysis showed that plasma  $\alpha$ -syn levels were negatively associated with urinary AD7c-NTP levels ( $r = 0.231$ ,  $P = 0.03$ ) (shown in **Figure 1**). However, no relationships were found between the plasma  $\alpha$ -syn levels and age ( $r = 0.146$ ,  $P = 0.176$ ), sex ( $Z = -1.03$ ,  $P = 0.303$ ), education ( $r = 0.044$ ,  $P = 0.683$ ), body mass index (BMI) ( $r = 0.145$ ,  $P = 0.179$ ), smoking ( $Z = -0.1$ ,  $P = 0.92$ ) and alcohol use ( $Z = -0.7$ ,  $P = 0.484$ ).

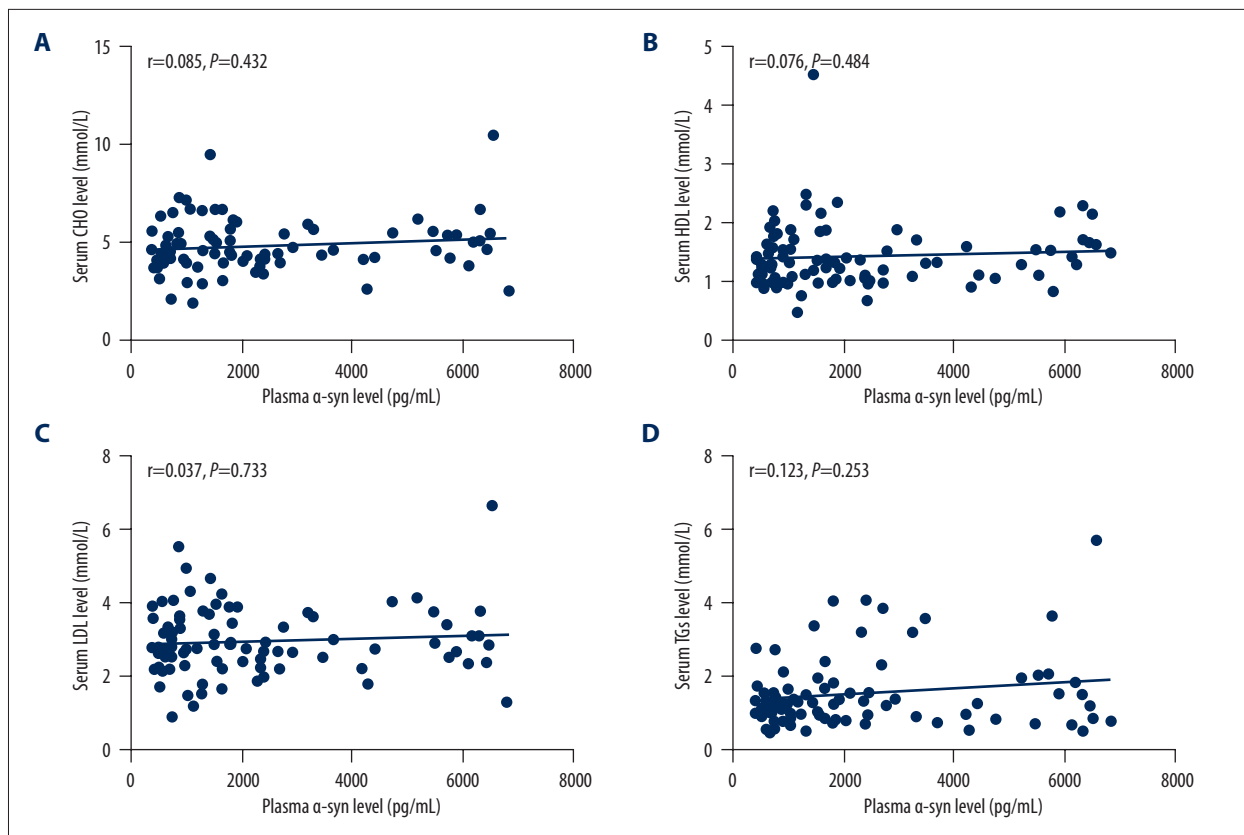
**Table 1.** Baseline characteristics of the AD and NC groups.

	AD (n=60)		NC (n=28)		P
Age (years)	70	(63, 77.5)	71	(62.25, 76)	0.989
Male (%)	20	(33.33%)	10	(35.71%)	0.826
Education (years)	11	(9,12)	9	(6.75, 12)	0.558
Plasma $\alpha$ -syn (pg/mL)	1879.16	(794.28, 4017.76)	993.67	(728.11,1627.26)	0.045
Urine AD7c-NTP (ng/mL)	0.90	(0.55, 1.74)	0.61	(0.39, 1.05)	0.017
ApoE $\epsilon$ 4 (%)	15	(25%)	4	(14.29%)	0.255
BMI (kg/cm <sup>2</sup> )	24.2	(21.26, 25.64)	24.01	(21.02, 26.5)	0.907
Smoking (%)	9	(15%)	6	(21.43%)	0.658
Alcohol (%)	9	(15%)	2	(7.14%)	0.489
CHO (mmol/L)	4.61	(4.08, 5.59)	4.65	(4.21, 5.48)	0.879
HDL (mmol/L)	1.35	(1.06, 1.66)	1.38	(1.12, 1.75)	0.479
LDL (mmol/L)	2.81	(2.39, 3.63)	2.9	(2.47, 3.5)	0.837
TGs (mmol/L)	1.35	(0.96, 1.95)	1.03	(0.81, 1.57)	0.109

Baseline data are presented as the median and interquartile ranges (IQRs). Two-group comparisons, such as the analysis of differences in baseline characteristics between AD and NC, were analyzed by the independent-samples Mann-Whitney U test (unpaired). AD – Alzheimer’s disease; NC – normal control; SD – standard deviation; AD7c-NTP :- Alzheimer-associated neuronal thread protein;  $\alpha$ -syn –  $\alpha$ -synuclein; ApoE – apolipoprotein E; BMI – body mass index; CHO – cholesterol; HDL – high-density lipoproteins; LDL – low-density lipoprotein; TGs – triglycerides.



**Figure 1.** (A-C) Relationship between plasma  $\alpha$ -syn and urinary AD7c-NTP levels. The figures were made using GraphPad Prism (version 8.0.2, GraphPad Software). AD7c-NTP – Alzheimer-associated neuronal thread protein;  $\alpha$ -syn –  $\alpha$ -synuclein.



**Figure 2.** (A-D) Relationships between plasma  $\alpha$ -syn levels and lipids. The figures were made using GraphPad Prism (version 8.0.2, GraphPad Software).  $\alpha$ -syn –  $\alpha$ -synuclein; CHO – cholesterol; HDL – high-density lipoproteins; LDL – low-density lipoprotein; TGs – triglycerides.

The concentration of urinary AD7c-NTP in the AD group was higher than that in the NC group ( $Z=-2.383$ ,  $P=0.017$ ) (shown in **Figure 1**). However, no relationships were found between the urinary AD7c-NTP levels and age ( $r=-0.065$ ,  $P=0.548$ ), sex ( $Z=-1.056$ ,  $P=0.291$ ), education ( $r=-0.095$ ,  $P=0.378$ ), BMI ( $r=-0.024$ ,  $P=0.827$ ), smoking ( $Z=-1.215$ ,  $P=0.224$ ) or alcohol use ( $Z=-0.24$ ,  $P=0.811$ ).

#### Relationship Between Plasma $\alpha$ -syn Levels and Neuropsychological Assessment Scale Scores

Spearman correlation analysis revealed that plasma  $\alpha$ -syn levels were negatively associated with MMSE scores ( $r=-0.283$ ,  $P=0.007$ ). However,  $\alpha$ -syn levels were not associated with MoCA scores ( $r=-0.158$ ,  $P=0.143$ ) nor ADL scores ( $r=-0.171$ ,  $P=0.11$ ).

#### Relationship Between Urinary AD7c-NTP Levels and Neuropsychological Assessment Scale Scores

Spearman correlation analysis revealed that urinary AD7c-NTP levels were negatively associated with MMSE scores ( $r=-0.355$ ,  $P=0.001$ ), MoCA scores ( $r=-0.272$ ,  $P=0.01$ ), and ADL scores ( $r=0.239$ ,  $P=0.025$ ).

#### Relationships Between Plasma $\alpha$ -syn and Urinary AD7c-NTP Levels and ApoE $\epsilon 4$

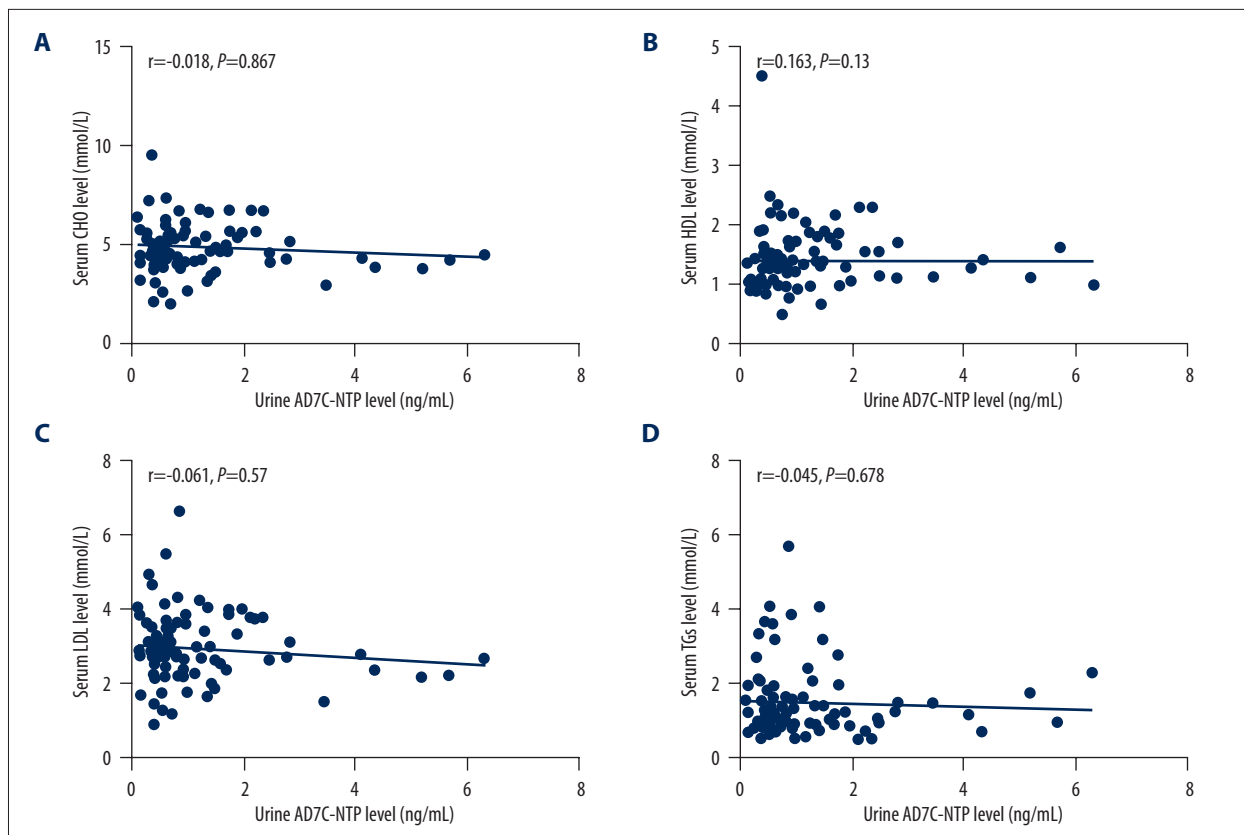
Two independent-samples Mann-Whitney U tests (unpaired) revealed that plasma  $\alpha$ -syn levels ( $Z=-0.147$ ,  $P=0.883$ ) and urinary AD7c-NTP levels ( $Z=-1.435$ ,  $P=0.151$ ) were not significantly different based on APOE  $\epsilon 4$  status.

#### Relationships Between Plasma $\alpha$ -syn and Urinary AD7c-NTP Levels and Lipids

Spearman correlation analysis revealed no relationship between plasma  $\alpha$ -syn levels and levels of CHO ( $r=0.085$ ,  $P=0.432$ ), HDL ( $r=0.076$ ,  $P=0.484$ ), LDL ( $r=0.037$ ,  $P=0.733$ ) or TGs ( $r=0.123$ ,  $P=0.253$ ) (shown in **Figure 2**).

Spearman correlation analysis revealed no relationship between urinary AD7c-NTP levels and levels of CHO ( $r=-0.018$ ,  $P=0.867$ ), HDL ( $r=0.163$ ,  $P=0.13$ ), LDL ( $r=-0.061$ ,  $P=0.57$ ), or TGs ( $r=-0.045$ ,  $P=0.678$ ) (shown in **Figure 3**).





**Figure 3.** (A-D) Relationships between urinary AD7c-NTP levels and lipids. The figures were made using GraphPad Prism (version 8.0.2, GraphPad Software). AD7c-NTP – Alzheimer-associated neuronal thread protein; CHO – cholesterol; HDL – high-density lipoproteins; LDL – low-density lipoprotein; TGs – triglycerides.

## Discussion

We found increased  $\alpha$ -Syn protein levels in patients with AD compared with NCs, which is unusual because this is a biomarker for Parkinson's disease (PD) [28]. At the beginning of the study, we first excluded patients with other neurological diseases, especially PD. All patients underwent MRI to rule out other brain diseases leading to cognitive decline. The subjects were independently recruited by 3 experienced doctors to ensure the correct diagnosis. While tau is a biomarker of AD and  $\alpha$ -syn is a pathological feature in PD and dementia with Lewy bodies (DLB), the presence of copathologies is important and very common in the above diseases [29].  $\alpha$ -Syn is a 140-amino-acid protein that is encoded by the alpha-synuclein gene (SNCA) and expressed at high levels in neuronal presynaptic terminals [30]. Nuclear magnetic resonance (NMR) spectroscopy experiments have shown that monomeric  $\alpha$ -syn can directly interact with tau variants through its highly negatively charged C-terminus, promoting amyloid fibrillation and accumulation in vitro [31]. Electron microscopy confirmed the immunopositivity of tubulin polymerization promoting protein (TPPP/p25) in postmortem AD brain tissue. TPPP/p25 was suggested to be a new marker of  $\alpha$ -syn [32]. These pathological features appear

not only in the human brain but also in peripheral fluids. CSF  $\alpha$ -syn levels are higher in patients with mild cognitive impairment (MCI) and AD than in healthy controls [33,34]. Sylvie Slaets's study showed that CSF  $\alpha$ -syn levels were significantly higher in AD than in synucleinopathies [33]. Vergallo's study showed that CSF  $\alpha$ -syn had positive correlations with both CSF t-tau and p-tau181 concentrations [35]. Filippo's study [6] showed that compared with NCs, AD patients had lower concentrations of  $\alpha$ -syn and its heterocomplexes, such as  $\alpha$ -syn/A $\beta$  and  $\alpha$ -syn/tau, in red blood cells. To the best of our knowledge, no study has compared  $\alpha$ -syn levels in human plasma between AD patients and NCs. In our study, AD patients had higher plasma  $\alpha$ -syn concentrations than NCs [medians and IQRs: 1879.16 (794.28, 4017.76) vs 993.67 (728.11,1627.26), respectively,  $P=0.045$ ]. Plasma  $\alpha$ -syn levels were also associated with MMSE scores ( $P=0.001$ ). Thus, although it is known to be associated with PD, it may also be a potential biomarker of AD. The pathological mechanisms should be explored in future studies.

AD7c-NTP is a transmembrane phosphate protein, and its molecular weight is approximately 41 kDa [36]. AD7c-NTP has high sensitivity and specificity in both urine and CSF. It was one of the potential biomarkers of early AD [37]. AD7c-NTP

immunoreactivity was found to co-localize with neurofibrillary tangles and dystrophic neurites [38]. Li's results showed that urinary AD7c-NTP levels in the subjective cognitive decline group ( $0.7561 \pm 0.5657$  ng/mL) were not different than levels in either depressive state ( $0.7527 \pm 0.5607$  ng/mL) or normal control ( $0.7214 \pm 0.5077$  ng/mL) groups [27]. However, in Zhang's study, urinary AD7c-NTP levels were significantly higher in the lower cognitive function group [ $0.48$  ( $0.21$ - $1.00$ ) ng/mL] than in the NC group [ $0.25$  ( $0.04$ - $0.44$ ) ng/mL;  $P < 0.001$ ] [39]. Lina's study showed that AD7c-NTP levels were higher in the AD group [median  $2.14$  (range  $0.49$ - $6.39$ ) ng/mL] and the MCI group [median  $1.57$  (range  $0.4$ - $4.15$ ) ng/mL] than in the normal group [median  $0.53$  (range  $0.04$ - $2.07$ ) ng/mL] [40]. Zhang's study showed that urinary AD7c-NTP levels were significantly higher in the lower cognitive function group [median  $0.48$  ( $0.21$ - $1.00$ ) ng/mL] than in the NC group [median  $0.25$  ( $0.04$ - $0.44$ ) ng/mL] [39]. In our study, urinary AD7c-NTP levels were significantly higher in AD patients [ $0.90$  ( $0.55$ ,  $1.74$ ) ng/mL] than in NCs [ $0.61$  ( $0.39$ ,  $1.05$ ) ng/mL]. A previous study showed that urinary AD7c-NTP levels were inversely correlated with MoCA [27] and MMSE [41] scores. Our study results are consistent with these results, which suggests that higher urinary AD7c-NTP levels may warn of proximal abnormalities in early AD patients. Our study also showed that AD7c-NTP levels were related to plasma  $\alpha$ -syn levels.

Another study of 329 cognitively normal right-handed Han Chinese subjects showed that urinary AD7c-NTP levels had a positive relationship with ApoE grade in the cognitively normal population and that urinary AD7c-NTP levels were significantly higher in subjects with ApoE  $\epsilon 4$  [ $0.6074$  ( $0.6541$ ) ng/mL] than in subjects without ApoE  $\epsilon 4$  [ $0.4368$  ( $0.3392$ ) ng/mL] [22]. ApoE is a cholesterol/lipid transporter in the central nervous system and is also known to be the most influential genetic risk factor for AD [42]. A study in mice showed that ApoE  $\epsilon 4$ , but not ApoE  $\epsilon 2$  or ApoE  $\epsilon 3$ , increased  $\alpha$ -syn pathology and astrogliosis. ApoE  $\epsilon 4$  also worsened neuronal and synaptic loss and impaired behavioral performance at 9 months of age [43]. However, in our study, we did not find any association with ApoE  $\epsilon 4$ . It may be that the sample size in our study was too small.

$\alpha$ -Syn can interact with lipoproteins in human plasma [44]. A study showed that hyperlipidaemia had a significant direct impact on tau protein deposition in the brain and damaged the blood-brain barrier [45]. Dyslipidaemia was shown to be a risk factor for AD [46]. Cédric Eichmann's study showed that the family of synucleins in humans, including mutants of the  $\alpha$ -syn family, can form HDL-like particles with different morphologies [47]. An 11-amino acid sequence from the ApoB protein LDL binding peptide can transport  $\alpha$ -syn, which is targeted by small interfering ribonucleic acid (siRNA), across the blood-brain barrier [48]. Higher low-density lipoprotein cholesterol (LDL-C) levels were associated with a higher risk of early-onset AD [49].

A study showed that urinary AD7c-NTP levels were also associated with human blood lipids [21]. Thus, we supported the notion that  $\alpha$ -syn had a relationship with AD through lipoproteins. However, in our study, we did not find any association with lipids, including CHO, HDL, LDL, and TGs. Therefore, the levels of AD7c-NTP and plasma  $\alpha$ -syn are not consistent with the levels of ApoE or lipids.

We first analyzed the relationship between  $\alpha$ -syn, AD7c-NTP, and ApoE. Further analysis was performed to determine whether they were associated with lipids in AD patients. This is a new clinical perspective in the diagnosis of AD. Currently, few biomarkers are used by neurologists in the clinic, and few studies have explored the relationship between them. All of the 3 biomarkers are related to A $\beta$  and tau, which are the hallmarks of AD. Moreover, we found that  $\alpha$ -syn levels are positively correlated with AD7c-NTP levels. Above, we speculate that  $\alpha$ -syn and AD7c-NTP, together, may be implicated in A $\beta$  and tau pathologies.

However, how  $\alpha$ -syn and AD7c-NTP are involved in AD pathogenesis and the interplay between them are still unclear. Large sample size studies, multicentre studies, and longitudinal studies should be performed for confirmation. In addition, a more elaborate case history of patients should be provided. The one limitation in the current study is that we quantified the protein levels of  $\alpha$ -syn and AD7c-NTP, but not ribonucleic acid (RNA) levels. Thus, whether  $\alpha$ -syn and AD7c-NTP's protein and RNA enrichment show consistency is still unknown, and this is an interesting question for future investigation. All the patients received a criterion standard biomarker-based diagnosis of AD and were matched for age and sex with the NC group in our study. Future studies may further investigate the interactions between  $\alpha$ -syn and AD7c-NTP.

## Conclusions

This study showed that the levels of plasma  $\alpha$ -syn protein and urinary AD7c-NTP were significantly increased in patients with AD compared with normal age-matched individuals, but not with ApoE alleles or serum lipid levels. Thus, plasma  $\alpha$ -syn levels may play an important role in the pathology of AD and this progression was not associated with lipids.

## Conflict of Interest

None declared.

## Institutional Review Board Statement

The study was reviewed and approved by our ethics committee and institutions (Ethics ID: 2017SY51). All subjects signed an informed consent form.

## Declaration of Figure Authenticity

All figures in our study have been created by the authors. We confirm that the images are original with no duplication and have not been previously published in whole or in part.

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