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RESEARCH ARTICLE

Plasma α-synuclein levels are increased in patients with obstructive sleep apnea syndrome

Hao-Lun Sun†, Bin-Lu Sun†, Dong-Wan Chen†, Yang Chen, Wei-Wei Li, Man-Yu Xu, Ying-Ying Shen, Zhi-Qiang Xu, Yan-Jiang Wang & Xian-Le Bu

Department of Neurology and Centre for Clinical Neuroscience, Daping Hospital, Third Military Medical University, Chongqing, China

Correspondence
Xian-Le Bu and Yan-Jiang Wang, Department of Neurology and Centre for Clinical Neuroscience, Daping Hospital, Third Military Medical University, Chongqing, China.
Tel: +86 23 68757850; Fax: +86 23 68711956; E-mails: buxianle@sina.cn; yanjiang_wang@tmmu.edu.cn

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†These authors contributed equally to this work.

Abstract

Objective: Obstructive sleep apnea syndrome (OSAS) is characterized by nocturnal intermittent hypoxemia and can increase the risk of Parkinson's disease. This study aimed to investigate the association between plasma α-synuclein levels and hypoxia in the patients with OSAS. **Methods**: We recruited 42 OSAS patients and 46 controls with simple snoring matched for age and gender. OSAS was diagnosed on the basis of the clinical symptoms as well as the nighttime polysomnography. Plasma total α-synuclein and phosphorylated α-synuclein levels were measured by ELISA kits. **Results**: The OSAS patients had significant higher levels of plasma total α-synuclein and phosphorylated α-synuclein levels. Both of the above indexes were positively correlated with the apnea–hypopnea index and the oxygen desaturation index, while they were negatively correlated with the mean and lowest oxyhemoglobin saturations. **Interpretation**: This study suggests that chronic intermittent hypoxia can increase the α-synuclein levels, which may contribute to the pathogenesis of Parkinson's disease.

Introduction

Parkinson's disease (PD) is a common disease in the middle-aged and elderly population, mainly affecting the motor system, as well as sleep problems and neuropsychiatric symptoms.[1] The presence of Lewy bodies, which were accumulated by the α-synuclein protein, is the major pathological process in PD.[2] Thus α-synuclein is considered to play a crucial role in the etiology and pathogenesis of PD. Many risk factors link to the etiology of PD.[3] Obstructive sleep apnea syndrome (OSAS), a prevalent disease characterized by recurrent episodes of upper airway obstruction, can lead to intermittent hypoxemia during sleep.[4] Recent researches found that OSAS was a risk factor for PD onset, and hypoxia may have contributed to it.[5,6] However, the detail mechanism remains to be further investigated. Previous studies both in vitro and in vivo reveal that hypoxia is able to induce overexpression of α-synuclein and its oligomer formation.[7–9] So our study is aimed to investigate the association between α-synuclein levels and hypoxia in OSAS patients.

Materials and Methods

Study participants

From September to December of 2014, we recruited 42 subjects diagnosed with OSAS from the Daping hospital. Among them, eight patients suffered with mild OSAS, 16 with moderate, and 18 with severe. Forty-six subjects with simple snoring were recruited as the controls matched for age and gender. The exclusion criteria included: (1) a

Table 1. Characteristics of the subjects.

	Controls (N = 46)	OSAS (N = 42)	P value
Age, years	43.46 (9.70)	45.02 (9.80)	0.453
Gender, male, n (%)	33 (71.74)	31 (73.81)	0.862
BMI, kg/m^2	26.10 (2.09)	26.96 (2.57)	0.126
Total sleep time, min	403.41 (52.15)	399.33 (41.58)	0.696
Sleep efficiency, %	84.84 (7.20)	84.51 (10.13)	0.864
AHI, events/h	2.22 (1.35)	33.96 (21.56)	<0.001
ArI, events/h	8.70 (4.25)	33.03 (17.02)	<0.001
ODI, events/h	2.14 (1.21)	28.07 (15.17)	<0.001
Mean SaO_2, %	95.65 (1.18)	90.64 (2.69)	<0.001
Lowest SaO_2, %	91.30 (1.66)	80.24 (6.38)	<0.001
Sleep stages 1 and 2, %	56.26 (10.24)	62.41 (15.76)	0.005
Sleep stages 3, %	25.73 (10.32)	25.50 (17.99)	0.197
REM, %	17.99 (5.67)	12.09 (6.83)	<0.001

Statistical comparisons were tested using two-tailed independent-sample t-tests, Mann–Whitney U test, or chi-squared test as appropriate. BMI, body mass index; AHI, apnea–hypopnea index; ArI, arousal index; ODI, oxygen desaturation index; SaO_2, oxyhemoglobin saturation; REM, rapid eye movement.

family history of PD; (2) other central nervous system disorders; (3) severe hepatic, renal, pulmonary, cardiac diseases, or neoplastic disorders; (4) long-term smoker or drinker. This study was approved by the Institutional Review Board of Daping Hospital (Chongqing, China).

The diagnosis of OSAS

OSAS was diagnosed on the basis of the clinical symptoms and the polysomnography (PSG) recordings as described in a previous study.[10] The apnea–hypopnea index (AHI) and oxygen desaturation index (ODI) could be measured based on the PSG recordings. Hypopnea was defined according to the American Academy of Sleep Medicine scoring manual[11,12]: (1) The peak signal excursions decreased by more than 30% from pre-event baseline using nasal pressure; (2) The duration of the decrease $\geq$ 30% in signal excursion is longer than 10 seconds; (3) There exists a $\geq$ 4% oxygen desaturation from baseline. Demographic data, including age, gender, weight, height, and educational levels, were gathered on admission. The medical histories of all subjects were also collected as described in our previous study.[13]

Blood sampling

To avoid the potential circadian rhythm effects, fasting blood was collected between 06:00 and 07:00. After drawn, the blood samples were immediately centrifuged and then reserved at −80°C. Before the acquisition of the blood samples, informed consent from each participant was obtained.

Measurements of plasma α-synuclein levels

Plasma total α-synuclein levels and phosphorylated α-synuclein levels were measured by human total alpha-synuclein ELISA Kit (Invitrogen) and phosphorylated alpha-synuclein ELISA Kit (LSBio). All measurements were carried out in accordance with the manufacturers' instructions. The standards and samples had received reduplicated measurements and analyses.

Statistical analysis

Statistical comparisons of demographic characteristics and plasma α-synuclein levels between OSAS patients and

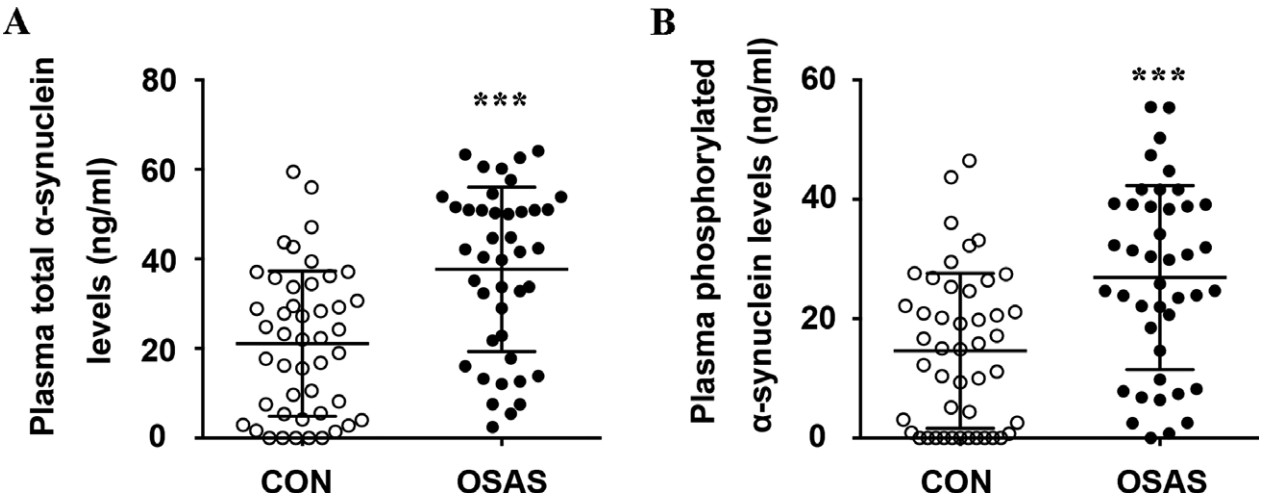

Figure 1. Comparison of the plasma total α-synuclein (A) and phosphorylated α-synuclein levels (B) between the controls and patients with obstructive sleep apnea syndrome (OSAS). *** $P < 0.001$.

Table 2. The partial correlation analyses of the plasma α-synuclein levels with AHI, ODI, mean SaO_2, and lowest SaO_2 in all subjects.

	Total α-synuclein		Phosphorylated α-synuclein	
	γ	*P*	γ	*P*
AHI, events/h	0.609	<0.001	0.610	<0.001
ODI, events/h	0.511	<0.001	0.486	<0.001
mean SaO_2	−0.502	<0.001	−0.514	<0.001
lowest SaO_2	−0.520	<0.001	−0.496	<0.001

Partial correlation analysis, adjusted for age, gender, body mass index, and comorbidities. AHI, apnea-hypopnea index; ArI, arousal index; ODI, oxygen desaturation index; SaO_2, oxyhemoglobin saturation.

controls were tested by two-tailed independent-sample t-tests, Mann–Whitney U test, or chi-squared test as appropriate. The correlations of plasma α-synuclein levels and AHI, ODI, $MSaO_2$ as well as $LSaO_2$ values were analyzed by partial correlation analyses. Data were shown as the mean ± standard deviation (SD). Statistically significant was defined by two-sided *P*-value less than 0.05. The data were analyzed with GraphPad Prism 6 (GraphPad Software, USA).

Results

Characteristics of the subjects

This study included 42 OSAS patients and 46 controls with simple snoring matched for age and gender (Table 1). No significant difference in body mass index (BMI) ($P = 0.126$), total sleep time ($P = 0.696$), and sleep efficiency ($P = 0.864$) was seen between the OSAS patients and controls. Compared with the controls, the OSAS group showed remarkably higher AHI ($P < 0.001$), ODI ($P < 0.001$), and arousal index (ArI) ($P < 0.001$). Also the OSAS group had dramatically lower mean SaO_2 ($P < 0.001$) and lowest SaO_2 ($P < 0.001$) than controls. And the significant differences between these two groups were also found in the proportions of stages 1 and 2 of sleep ($P = 0.005$) and rapid eye movement (REM) sleep ($P < 0.001$), but not in the stages 3 sleep ($P = 0.197$).

Plasma α-synuclein levels in the participants

As the Figure 1 showed, OSAS patients had significant higher levels of plasma total α-synuclein (37.68 ± 18.35 ng/mL vs. 21.08 ± 16.21 ng/mL, $P < 0.001$) and phosphorylated α-synuclein (26.87 ± 15.42 ng/mL vs. 14.61 ± 12.98 ng/mL, $P < 0.001$) levels than the controls.

Correlations of plasma α-synuclein levels with AHI

We used the partial correlation analyses to analyze the relationships between plasma α-synuclein levels and AHI by adjusting for age, gender, and BMI. In all participants, both of total α-synuclein and phosphorylated α-synuclein levels in plasma were positively correlated with AHI (Table 2). In the OSAS patients, the plasma levels of total α-synuclein ($\gamma = 0.550$, $P < 0.001$) and plasma phosphorylated α-synuclein ($\gamma = 0.613$, $P < 0.001$) levels were also positively correlated with AHI (Fig. 2).

Correlations of plasma α-synuclein levels with ODI, mean SaO_2, and lowest SaO_2

Then, we analyzed the associations between plasma α-synuclein levels and the extent of hypoxia. In all participants, the plasma total α-synuclein and phosphorylated

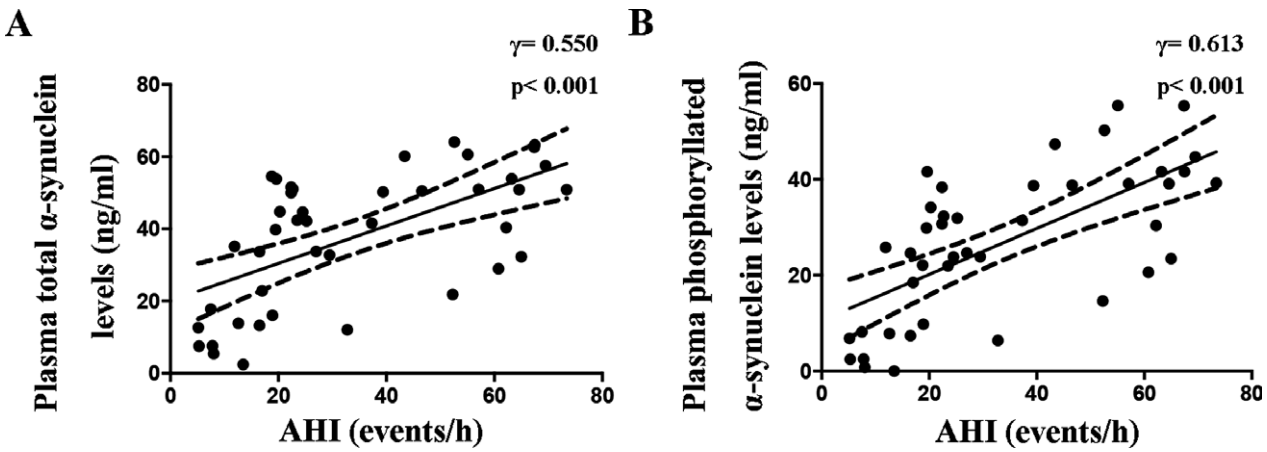

Figure 2. Partial correlations of the plasma total α-synuclein levels (A) and phosphorylated α-synuclein levels (B) with AHI in the patients with obstructive sleep apnea syndrome adjusted for age, gender, BMI, and comorbidities.

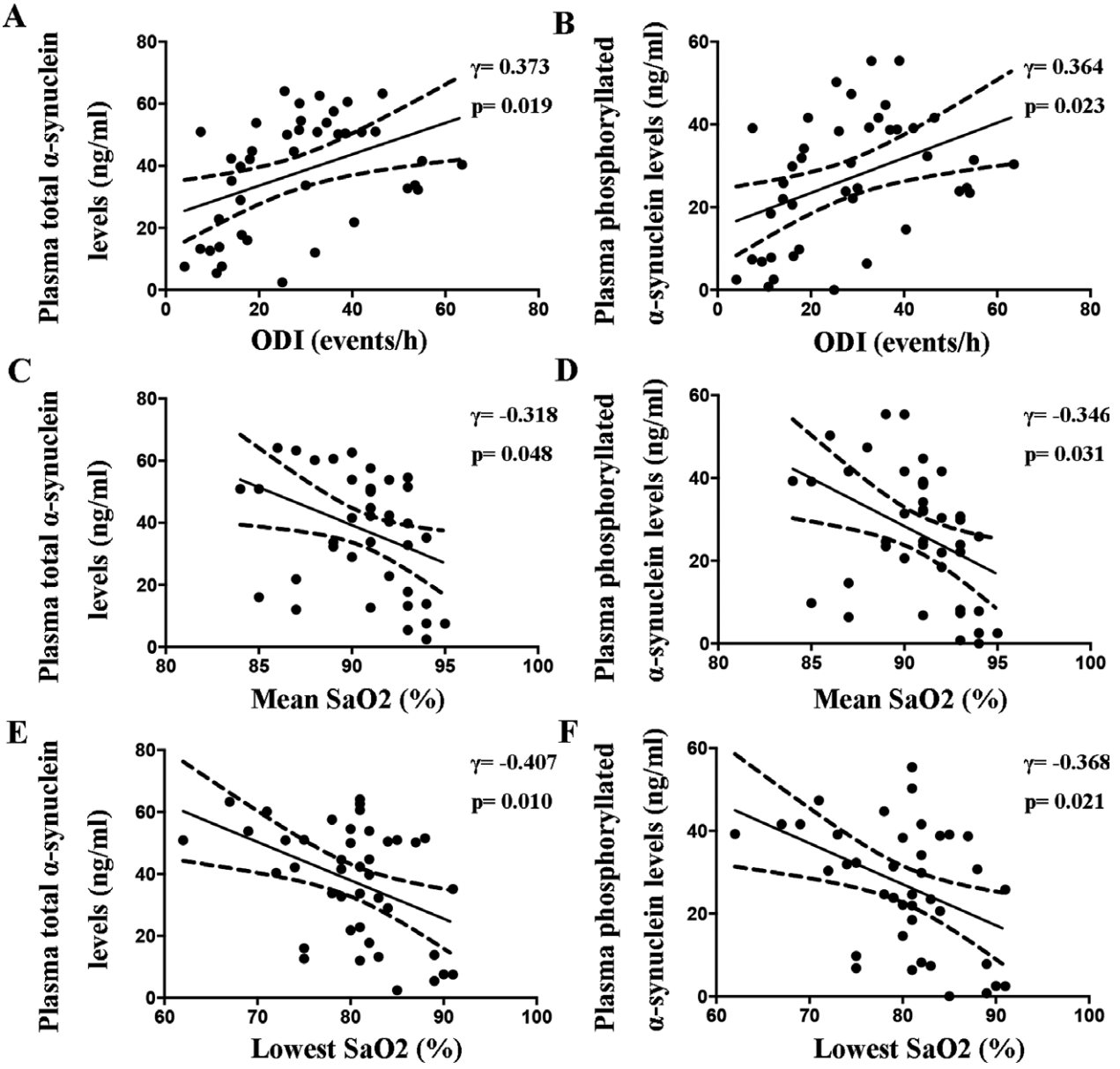

Figure 3. Partial correlations of the plasma α-synuclein levels with the ODI (A and B), mean SaO_2 (C and D), and lowest SaO_2 (E and F) in the patients with obstructive sleep apnea syndrome adjusted for age, gender, BMI, and comorbidities.

α-synuclein levels were positively correlated with ODI, while negatively correlated with mean SaO_2 and lowest SaO_2 (Table 2). In OSAS patients, the plasma total α-synuclein (γ = *0*.373, P = 0.019) and phosphorylated α-synuclein (γ = *0*.364, P = 0.023) levels were both positively correlated with ODI (Fig. 3A and B). In addition, the plasma total α-synuclein ($\gamma = -0.318$, P = 0.048) and plasma phosphorylated α-synuclein ($\gamma = -0.346$, P = 0.031) levels were both negatively correlated with mean SaO_2 (Fig. 3C and D). We also found negative correlations of the lowest SaO_2 with plasma total α-synuclein ($\gamma = -0.407$, P = 0.010) and phosphorylated α-synuclein ($\gamma = -0.368$, P = 0.021) levels (Fig. 3E and F). The above results suggested that the plasma α-synuclein levels were associated with the degree of hypoxia.

Discussion

In the present study, we for the first time found that the OSAS patients had significantly higher plasma total α-synuclein and phosphorylated α-synuclein levels. Besides, both of them were positively correlated with

disease severity and the degree of hypoxia. These results suggest that hypoxia may be involved in the pathogenesis of PD.

Recent epidemiological studies found that there was a significantly elevated risk of developing PD in the patients with OSAS,[5,6] especially in female patients with OSAS.[14] On the other hand, PD patients exhibited a high prevalence of OSAS (20%–60%), which may be related to specific phenotype and rapid progression of PD.[15–17] Thus, the cause and effect of α-synuclein levels changes in OSAS require further investigation. Recent researches suggested that OSA and REM sleep behavior disorder can manifest with similar symptoms and coincide in a certain number of cases.[18–20] So in OSAS, except for nocturnal intermittent hypoxia, REM sleep behavior disorder may also be associated with the pathophysiology of the disease and involved in the development of α-synucleinopathy.[21,22] These further indicated that OSAS may aggravate the pathological process in early stage of PD.

Alpha-synuclein, abundant in the brain, is composed of 140 amino acids and encoded by the SNCA gene.[23] The aggregation of α-synuclein forms Lewy bodies, which is the typical pathological feature of PD.[2] Although the function of α-synuclein is still not well understood, studies have shown that α-synuclein plays a essential role in the pathogenesis of PD.[24,25] It is related to apoptosis suppression, glucose regulation, and the modulation of calmodulin activity.[26] The phosphorylation of α-synuclein may accelerate this process by ubiquitinated and the disruption of internalized vesicle membranes.[27,28] In addition, plasma levels of total α-synuclein and phosphorylated α-synuclein are increased in PD and have been thought to be potential biomarkers of disease progression.[29–31] Whether peripheral α-synuclein has pathogenic capacity remains unclear. Previous study found that peripheral injection of α-synuclein was able to induce brain α-synucleinopathy and a motor phenotype in mice.[32] So the elevated α-synuclein levels in OSAS patients in the present study indicate that OSAS may facilitate the initiation of PD pathogenesis.

In the brain, α-synuclein is highly expressed in the neurons. Additionally, α-synuclein is also expressed in various peripheral tissues such as the skin, liver, kidney, adrenal gland, and so on.[33,34] A previous study quantified α-synuclein levels in the different fractions of blood and found that more than 99% of α-synuclein was from the red blood cells with less than 1% of the total α-synuclein from the platelets and peripheral blood mononuclear cells.[35] The oligomeric α-synuclein in red blood cells can be a potential diagnostic biomarker for PD.[36,37] It has been shown that short-term or mild hypoxic stress is able to cause α-synuclein overexpression and reduce the cell viability in HEK293T cells.[7,9] Studies in vivo also revealed that the α-synuclein level was significantly increased in rat cortex under hypoxic conditions.[8] However, more studies are needed to prove the effect of hypoxia on α-synuclein expression in animal model and humans. At this point, our findings are consistent with those findings that hypoxia is related to increased production of α-synuclein. The systemic inflammation in OSAS can change the function and properties of red blood cells, which may also contribute to the increased expression of α-synuclein.[38] Additionally, oxidative stress can promote the uptake of extracellular α-synuclein in oligodendrocytes, accelerating the accumulation and oligomerization of it.[39]

Continuous positive airway pressure (CPAP) is currently thought to be the standard treatment for OSAS. Recent researches have shown that CPAP can alleviate sleep problems and retard cognitive decline in PD patients.[40,41] However, whether this benefit is associated with the decrease in α-synuclein levels by CPAP remains unknown. Further prospective studies are needed to explore whether CPAP can reduce plasma α-synuclein levels in OSAS patients and delay the course of PD.

However, our study has some limitations. First, as this study used a cross-sectional design, we were unable to determine the cause and effect of hypoxia and α-synuclein. In addition, the confounders such as sleep disruption may also affect the data. Moreover, more prospective studies with larger sample size are required to explore the clinical significance of elevated plasma α-synuclein in OSAS patients for predicting the risk of developing into PD.

In summary, the present study found that increased α-synuclein levels in the plasma are correlated with the degree of hypoxia in OSAS, indicating that chronic hypoxia caused by OSAS may be involved in the pathogenesis of PD.

Acknowledgments

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Conflicts of Interests

The authors declare no financial or other conflicts of interests.

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