Serum phosphorylated α-synuclein: A potential biomarker of poststroke cognitive impairment

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Research Article

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Abstract

Background

Ischemic stroke (IS) is characterized by cerebral infarction caused by acute cerebral vascular occlusion, with high rates of morbidity, mortality, and disability. Poststroke cognitive impairment, one of the major secondary impairments, is associated with greatly reduced quality of life in many patients with IS. Because poststroke cognitive impairment has insidious onset and progressive progression, the development of early warning biomarkers is essential for this disease.

Methods

In this study, we detected phosphorylated α-synuclein (p-α-syn) pathology in the brain of distal middle cerebral artery occlusion (dMCAO) mice and hypoxia mice. We collected blood samples and routine biochemistry data of patients with IS and those who underwent physical examination in Beijing Boai Hospital from July to December 2021. We examined the serum level of p-α-syn in those people by ELISA.

Results

The level of p-α-syn was significantly increased and showed pathological aggregation around the cerebral infarct in dMCAO mice. And the similar aggregation in neurons were also observed in the brain of chronic hypoxia mice, thus suggesting that hypoxia is the internal cause of α-syn pathology. The serum level of p-α-syn in patients with IS was significantly lower than that of control group, and this lower serum level was positively correlated with the cognitive level of these patients. Further association analysis revealed that the decrease in the serum high-density lipoprotein level of patients with stroke was significantly correlated with their decreased p-α-syn level.

Conclusions

Serum p-α-syn has the potential to serve as a biomarker for poststroke cognitive impairment.

Introduction

As the most common cerebrovascular disease, ischemic stroke (IS) is caused by acute ischemic hypoxia and brain tissue necrosis induced by cerebrovascular stenosis or occlusion, and it is characterized by high rates of morbidity, mortality, and disability[1]. Following the onset of stroke, cognitive impairment associated with stroke occurs in many patients[2] and can be classified as vasogenic, neurodegenerative, or mixed [3]. Stroke also increases the incidence of cognitive impairment by 5 to 8 fold[4]. The occurrence of cognitive impairment can greatly reduce the quality of life of patients with stroke. Moreover, the time of onset of poststroke cognitive impairment is also inconsistent and ranges from 1 month to several years.
after stroke, with the characteristics of insidious onset and progressive progression [5] [6] [7]. Therefore, effective indicators for early warning of poststroke cognitive impairment are urgently required.

The α-synuclein (α-syn) is a protein closely associated with neurodegeneration and cognitive damage[8]. The α-syn is involved in various biological processes such as synaptic vesicle release and transmission of neurotransmitters under physiological conditions[9] [10]. In a pathological state, α-syn is prone to phosphorylation at serine 129 (p-α-syn), resulting in an abnormal aggregation of α-syn, which leads to neuronal damage [11] [12]. Diseases with abnormal α-syn pathology are collectively referred to as synucleinopathy[13], such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB), which mainly show progressive neurodegeneration, with varying degrees of cognitive impairment in the middle and late stages of the disease[14]. Several clinical studies have also shown a relationship between stroke onset and synucleinopathy[15] [16] [17]. Following stroke, the damaged cerebral environment is spontaneously restored; however, it is usually compensatory and incomplete[18], and secondary neurodegeneration inevitably occurs after stroke[19]. The α-syn and p-α-syn levels were significantly increased in animal models of IS[20], and the inhibition of α-syn significantly reduced brain injury and improved neurological function, thus indicating its important role in secondary brain injury following stroke[21] [22] [23] [24]; however, the specific mechanisms remain unclear.

Although α-syn was previously considered to be a neuronal protein, in recent years, α-syn has been detected in cerebrospinal fluid, blood, and saliva[25] [26]. α-syn and p-α-syn levels have been considered using in the early diagnosis of synucleinopathy such as PD and DLB[27] [28] [29], and α-syn heteromers can play an important role in differencing neurodegenerational cognitive impairment[30] [31]. Recent clinical studies have also shown that the α-syn level in red blood cells of patients with acute stroke is significantly higher than that of the control group[32]. However, it remains to be confirmed whether α-syn can be used as a biomarker of poststroke cognitive impairment.

In the present study, we found an increase and aggregation of p-α-syn in the brain of IS model mice and hypoxic model mice, and these abnormal pathologies of α-syn were colocalized with the neuronal injury. We also found that serum p-α-syn levels decreased significantly in patients with IS, which was closely related to the occurrence of cognitive impairment after stroke. p-α-syn levels were positively correlated with high-density lipoprotein (HDL) levels in the serum of patients with IS. In conclusion, our present study found a positive correlation between serum p-α-syn levels and poststroke cognitive function, thus suggesting that p-α-syn levels can be used as a potential biomarker of poststroke cognitive impairment.

**Methods And Materials**

1. **Animals**

Adult male C57BL/6 mice aged 2–3 months were used for the present study. All animals were housed under controlled conditions with a 12-h light/dark cycle, 22 ± 3°C temperature, and 60% ± 5% humidity. The mice were kept in captivity for at least 1 week before the experiment. All mice were given free access
to water and a standard rodent diet. All animal experiments were approved by the Animal Care and Use Committee of the Institute of Animal Management, Capital Medical University (Approval Number: AEEI-2021-058) and conducted in accordance with the ethical requirements.

1.1 Hypoxic treatment: Mice were administered 13% oxygen at normal temperature and pressure in a closed hypoxia chamber (China Innovation Instrument Co., Ltd, Ningbo, Zhejiang, China). The bedding and hypoxic chamber were cleaned once a week, and the hypoxic chamber was kept dry and clean daily by using a desiccant and CO₂ adsorbent.

1.2 The dMCAO model: Focal cortical ischemia was induced in mice, and their body temperature was maintained at 37°C ± 0.5°C. Under isoflurane anesthesia (induction dose: 3%; maintenance dose: 1.5%), the left common carotid artery was ligated, and the distal striatum branch of the left middle cerebral artery was electrocoagulated[33]. The exclusion criteria were as follows: intracerebral hemorrhage or subarachnoid hemorrhage during the operation, death before the observation time point, and blood flow decreased by < 75% according to laser speckle imaging after electrocoagulation. The sham operation was performed in the same manner without distal occlusion of the middle cerebral artery and the common carotid artery.

2. Study Population

In this study, blood samples were collected from patients diagnosed to have IS and those who underwent physical examination in Beijing Boai Hospital from July to December 2021. The control group had no history of stroke, but they might have hypertension or diabetes. This research project complied with the ethical review of the scientific research project of the Medical Ethics Committee of China Rehabilitation Research Center [Project Number: CRRC-IEC-EF-SC-005-01].

Baseline parameters for the study population were recorded, including age, sex, systolic blood pressure, diastolic blood pressure, smoking history, and routine biochemistry data.

3. Behavior Test

3.1 Garcia Score: The highest score was 18 points when the neuronal function was normal, and the lowest score was 3 points when the neuronal function was severely damaged. (1) Autonomic activity: movement and touching at least three sides of the cage wall – 3 points; movement and touching at least one side of the cage wall – 2 points; little movement – 1 point; no autonomic activity – 0 points. (2) Body symmetry: bilateral body symmetry – 3 points; the affected side movement was slow: 2 points; the affected side showed slight movement: 1 point; the affected side showed no movements: 0 points. (3) Forelimb stretching: lateral stretching symmetry – 3 points; affected side forelimb stretching, but less than the normal side – 2 points; affected side forelimb slight stretching – 1 point; affected side forelimb no stretching – 0 points. (4) Ability to climb and hold the iron cage: easy climbing; 3 points for strong holding; 2 points for damage on the affected side; 1 point for inability to climb or turn. (5) Sensory
response of the body: the response of both sides is the same − 3 points, the response of the affected side is less than that of the normal side − 2 points, no reaction − 1 point. (6) For the reaction to facial hair touching, the reaction on both sides was equal − 3 points, the reaction on the affected side was lower than that on the normal side − 2 points, and the affected side showed no reaction − 1 point[33].

3.2 Grid-walking test: The test animals were placed on the water grid (5 × 5 cm per unit) and allowed to walk freely. Behavioral data such as the total number of steps and the number of hind paw slips and misses were recorded within 1 min[34].

3.3 Adhesive removal test: An adhesive tape was wound around the palms of each front paw of the animal, and the contact time and the time to remove the tape was measured. The test was repeated three times at an interval of more than 5 min [35].

3.4 Rotarod test: An accelerated rotarod test was used to evaluate sensorimotor deficits in mice. Before the test, the mice were placed on a rotating rod and allowed to practice for 3–5 days. After the initiation of hypoxia, the mice were tested once a week on a rotating stick that accelerated from 4 rpm to 40 rpm in 5 min for three times. The result was recorded as the duration for which the mice could stay on the pole before falling off[36].

3.5 Novel object recognition test: The formula to calculated recognition index (RI) is as follows: \( RI = \frac{\text{new object}}{\text{new object} + \text{old object}} \times 100\% \). The mice were acclimatized to the environment before training and testing. During the training, two objects, A and B, were placed on the left and right ends of a side wall, respectively, and the mice were placed in the field with their backs facing the two objects at the same distance. After adaptation, the contact between the mice and the two objects was recorded, including the number of times of touching the object and the time spent exploring. After 10 min, the mice were placed back in the original box, and the test was performed after the mice rested for 1 h. Object B in the field was changed to object C, and the mice were again placed with their back facing the two objects. The same distance was maintained between the two objects, and the observation was performed for 2–5 min. A video recording equipment was used to observe the mice and their exploration of the new and old objects[37].

4. Immunofluorescence Staining

The heart was perfused with 0.01M phosphate-buffered saline and 4% paraformaldehyde. After gradient dehydration with 20–30% sucrose, 20-µm coronal sections were made with a frozen slicer. After routine antigen repair, drilling, and antigen sealing, the sections were incubated with the following primary antibodies overnight: MAP2 (1:500, Abcam, ab32454), a-syn (1:500, Santa Cruz Biotechnology, sc-69977), p-a-syn (1:1:W, Wako, 015-25191), and P-A-SYN (1:500, Wako, 015-25191). The sections were then incubated with secondary antibodies (1:500, Alexa Fluor 488 or 594, Jackson ImmunoResearch) at room temperature for 1 h. Immunofluorescence images were acquired by confocal laser scanning microscopy (LSM880, Zeiss, Gottingen, Germany, or SP8 X, Leica) after nuclear re-staining with Hoechst stain.
5. Infarct Volume Measurement

Mice were sacrificed after paraformaldehyde perfusion, and their brains were collected and stained to determine the infarct volume. The infarct volume was calculated by subtracting the noninfarct area of the affected cerebral hemisphere from the area of the healthy cerebral hemisphere to obtain the infarct area of the single cerebral slice and then adding the infarct area of each cerebral slice to obtain the infarct volume.

6. ELISA

Standard antigen and blood samples to be tested were diluted to appropriate proportions. The standard antigen and blood samples were diluted to the specified concentration with CBS and stored in a refrigerator at 4°C. The diluted antigen was added to the ELISA plate at the volume of 100 µL/well and placed in a refrigerator at 4°C overnight (16 h). After washing the plate, 5% BSA was added for sealing the plate for 2 h. After washing, the detection antibody p-α-syn (Abcam, ab51253) was added (100 µL/well) to the plate at 1:200,000 dilution with PBST, and the plate was incubated at 37°C for 2 h. After washing the plate, IgG labeled with alkaline phosphatase (mAP-IgG, Sigma, E2636; 1:1000 diluted with PBST) was added, and the plate was incubated at 37°C for 1 h. The plate was washed, and the AP-acting substrate pNPP (SurModics, PNPS-1000-01) was added. The plate was incubated at 37°C in dark. Sweep plate of enzyme marker, parameter set as 405 nm, 0.1s.

7. Statistical Analyses

All raw data were recorded and saved using Excel. Statistical data were analyzed using GraphPad Prism Software (GraphPad Software, Inc.). All results were analyzed by a t-test, one-way ANOVA, and a contingency table chi-square test. Data were expressed as mean ± SEM or mean ± SD, and p < 0.05 was considered statistically significant. The heat map was plotted using the R package (version 4.2.1). The source code of the corrplot package was slightly modified to improve the layout, and some features were added.

Results

1. Neurological impairment and aggregation of p-α-syn around the infarct area were observed in IS mice.

We developed an IS mice model by performing the classic distal middle cerebral artery occlusion (dMCAO) surgery and detected cerebral blood flow by laser speckle imaging. The results showed that cerebral blood flow on the infarct side of mice in the dMCAO group was significantly reduced as compared to that on the opposite side (Fig. 1A, B). Moreover, Garcia score, grid-walking test, and adhesive removal test were used to evaluate the neurological function. Garcia score of the dMCAO group decreased as compared to that of the Sham group, thus indicating that dMCAO surgery led to neurological dysfunction in mice (Fig. 1C). The results of the grid-walking test showed that the foot fault
rate of mice in the dMCAO group was significantly increased as compared to that in the Sham group, thus indicating that motor coordination of mice decreased after dMCAO surgery (Fig. 1D). The adhesive removal test revealed that the time of touching and removing the paper in the dMCAO group was significantly extended as compared to that in the Sham group, thus indicating that dMCAO impaired the sensorimotor function of mice (Fig. 1E, F). These results suggested that the dMCAO group mice showed significant neurological function disorders as compared to the Sham group mice. Staining of the neuronal marker Map2 was performed on the brain sections of mice, and the results showed significant infarct areas in the dMCAO group (Fig. 1G). The infarct volume was calculated according to the color rendering results of Map2, and the average infarct volume was 10.56 mm$^3$ (Fig. 1H). These results indicated that dMCAO surgery caused neurological damage and cerebral infarction in mice.

To determine the p-α-syn level, immunofluorescence staining was performed on the brain tissue slices of mice in the sham and dMCAO groups. The results showed that the p-α-syn level around the infarct area of the dMCAO group mice was significantly increased and p-α-syn was aggregated as compared to that in the sham group mice, while the Map2 level was significantly decreased. This finding suggested that the peri-infarct nerve injury was associated with pathological p-α-syn deposition (Fig. 1I).

2. Neuronal injury and p-α-syn aggregation in cortical neurons were observed in hypoxic mice.

The pathological changes caused by ischemia are mainly due to the reduced utilization of oxygen in the body and the formation of a hypoxic internal environment. Therefore, we speculated that hypoxia was the internal cause of induction in the increase and aggregation of p-α-syn. Hence, we constructed a whole-body hypoxic mice model by using a hypoxic chamber with 13% O$_2$ conditioning for 2 weeks (Fig. 2A). According to the rotarod behavioral test and the novel object recognition test, 7 days of persistent hypoxia resulted in cognitive impairment of mice, and 14 days of persistent hypoxia further led to significant impairment of motor function (Fig. 2B, C). Nissl staining was performed on brain tissue slices from different groups of mice to observe neuronal damage. After 7 days of hypoxia, the Nissl particles were blurred, the margin was not clear, and the number of neurons decreased significantly (Fig. 2D, E). Immunofluorescence staining of Map2 also showed that the p-α-syn level in cortical neurons of mice increased significantly due to hypoxia. p-α-syn gradually accumulated in the neurons with the increase in hypoxia time (Fig. 2F) and was negatively correlated with the Map2 level (Fig. 2H). These results suggested that hypoxia induced phosphorylation and aggregation of α-syn in the brain, which may be an important cause of hypoxia-induced neuronal injury.

3. Serum p-α-syn levels were significantly decreased in patients with IS and were correlated with poststroke cognitive impairment.

The abovementioned results show that ischemia and hypoxia induced an increase and aggregation of p-α-syn in brain tissues. Next, we observed the relationship between stroke incidence and p-α-syn level in samples from patients with IS. We collected serum samples from patient with IS and the control group. Basic information (Table 1) and medical history details (Table 2) of all subjects were as follows.
Serum samples collected from each group were subjected to p-α-syn level detection by ELISA. The results showed that the serum p-α-syn level was significantly decreased in stroke patients as compared to that in the control group (Fig. 3A), and the area under the curve (AUC) value of the stroke group was 0.7207 [95% confidence interval (CI): 0.6485–0.7929]. These results indicated that the serum p-α-syn level had the potential to serve as a biomarker for differentiating IS (Fig. 3B).

We further analyzed the correlation between serum p-α-syn levels with onset time, medical history, stroke history, and family history in patients with IS (Table 2). In terms of stroke onset time, serum p-α-syn levels decreased significantly in 30–60 days after the onset of IS and subsequently recovered (Fig. 3C). However, factors such as diabetes, hyperlipidemia, hypertension, stroke history, NIHSS score, smoking history, and drinking history did not significantly alter the serum p-α-syn level (Fig. 3D-I).
To determine whether changes in serum p-α-syn levels indicated the occurrence of poststroke cognitive impairment, we further analyzed the correlation between serum p-α-syn levels with cognitive-related clinical manifestations. Serum p-α-syn levels in IS patients without cognitive impairment were significantly lower than those in the control group. However, serum p-α-syn levels tended to decrease further in IS patients with cognitive impairment (Fig. 4A). Similar results were obtained after the MMSE score test. An MMSE score lower than 27 points often reflects poststroke cognitive impairment[38]. The serum p-α-syn level decreased with the exacerbation of cognitive impairment (Fig. 4B). Serum p-α-syn levels for differentiating control from poststroke cognitive impairment had an AUC value of 0.7660 [95% CI: 0.6798–0.8523], suggesting that serum p-α-syn was a stronger biomarker for poststroke cognitive impairment (Fig. 4C). In conclusion, serum p-α-syn could be used as a biomarker for early warning of poststroke cognitive impairment.

4. Decrease in the serum p-α-syn level in patients with stroke is associated with the decrease in HDL levels.

We further analyzed the correlation between serum p-α-syn levels with the biochemical test results of patients with IS (Table 3). First, we focused on lipid metabolism-related indicators and found a significant reduction in the levels of HDL, low-density lipoprotein (LDL), and total cholesterol (TC) in patients with IS. Statistical analysis of liver function indicators revealed no changes in the levels of creatinine (Cre), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and direct bilirubin (Dbil). In contrast, the levels of prealbumin (PA), lactate dehydrogenase (LDH), total bile acid (Tbil), and total bilirubin (TBA) were significantly decreased in patients with IS. Statistical analysis of kidney function-related indicators showed no difference in urea levels between the IS and control groups, while the uric acid (UA) level decreased significantly in patients with IS. Moreover, no difference was observed in the blood glucose level between the IS and control groups. Total protein (TP) level was significantly reduced in the IS group. In summary, patients with IS showed changes in several biochemical indicators.
Next, a correlation analysis was performed to determine whether biochemical indicators with significant changes were associated with the decrease in the serum p-α-syn level in patients with IS. Pearson's correlation between each indicator is shown in the form of a heat map. The number represents Pearson's correlation coefficient ($r$) that measures the strength and direction of the relationship between two indicators and has a value between $-1$ and $1$ ($|r|<0.3$ = weak correlation, $0.3–0.5$ = medium correlation). The reliability of correlations is indicated by asterisks (*$p<0.5$, **$p<0.05$, ****$p<0.001$). As shown in Fig. 5, both TBA and UA levels were weakly and negatively correlated with the p-α-syn level ($r_{TBA} = 0.1$, $r_{uric acid} = 0.1$). A medium and positive correlation was observed between HDL and p-α-syn levels ($r_{HDL}= 0.32$) (Fig. 5). These results indicated a significant correlation between p-α-syn and HDL levels. We next determined the relationship between the HDL level and poststroke cognitive impairment. Patients with IS were further divided into the normal HDL group (IS-HDL (N)) and the lower abnormal HDL group (IS-HDL (L)), and their serum p-α-syn levels were compared. The results showed that serum p-α-syn levels were also significantly decreased in IS-HDL (L) group (Fig. 6A). Linear regression for the overall population showed a positive correlation between HDL and p-α-syn levels (Fig. 6B); although HDL in the control
Discussion

In the present study, we found that p-α-syn aggregation occurred around the infarct area in IS mice, and hypoxia was the intrinsic cause of α-syn pathology. Data from clinical studies showed that serum p-α-syn levels were significantly reduced in patients with IS, and this was positively correlated with cognitive function and HDL levels of patients with IS. These results suggested that serum p-α-syn could be used as a biomarker of poststroke cognitive impairment, thus providing a new perspective on its early diagnosis.

Our study results suggested that the decline in the serum p-α-syn level could be used as a biomarker for cognitive impairment after IS. Recent studies have shown a high incidence of poststroke cognitive impairment, ranging from 24–70% [5] [39] [40]. Some studies have also focused on the risk factor or predictive biomarkers of poststroke cognitive impairment, such as blood levels of Hcy, CRP, TC, LDL-C and Hb[41] [42]. miRNA is also a common diagnostic marker. miR-132 is a risk marker for poststroke cognitive impairment[43]. miR-let-7i is also believed to be overexpressed in the serum of patients with poststroke cognitive impairment and can be used as a diagnostic biomarker for this condition[44]. Previous studies have shown that high serum levels of neurofilaments (NfL), neuron-specific structural proteins, can also be used as a predictive biomarker for the development of cognitive impairment 90 days after IS [45]. Similarly, the present study showed that α-syn, as a presynaptic protein, changed to its pathological form (p-α-syn) in serum, and it could also be used as a biomarker of poststroke cognitive impairment.

In the present study, the brain of model mice showed an increased level and deposition of p-α-syn, together with neuronal damage. Interestingly, we also found a significant decrease in serum p-α-syn levels in patients with IS. The α-syn can be transported out of the brain along the artery wall[46]; hence, we hypothesized as follows: under normal conditions, as a self-protection mechanism, the body can actively exude toxic p-α-syn from the brain into the peripheral blood. However, when an IS occurs, this self-protection mechanism fails to exert its protective effect, and the p-α-syn level in the peripheral blood decreases. According to some studies, energy or oxygen deficiency caused by acute vascular obstruction damages specific areas in the brain and ultimately leads to progressive cognitive impairment after IS[47]. Our previous studies have shown that long-term hypoxia can damage nerve function and promote the transformation of α-syn into p-α-syn and its subsequent accumulation in the brain[48] [49]. In the present study, we found nerve damage and p-α-syn accumulation around the damaged area in the brain of both IS and simple hypoxia injury model mice. Therefore, we speculated that excessive toxic p-α-syn was rapidly accumulated during IS and could not be cleared by the above self-protective mechanism. Consequently, the serum p-α-syn level was decreased.
In the present study, we found a positive correlation between serum HDL and \( p-\alpha \)-syn levels. IS patients with a low HDL level had a higher incidence of cognitive impairment. Synucleinopathy and stroke may have a common vascular risk factor, i.e., dysregulation of lipid metabolism\[^{17}\] [\(^{50}\)]. Previous studies have confirmed that the HDL level is negatively correlated with IS incidence\[^{51}\] [\(^{52}\)]. Changes in sphingolipid metabolism have also been detected in the cerebrospinal fluid and blood of patients with synucleinopathy [\(^{53}\)]. Members of the synuclein family can produce different forms of HDL-like particles\[^{54}\], and \( \alpha \)-syn can interact with apolipoprotein in blood\[^{55}\]. However, further studies are required to understand the specific mechanisms of interaction between \( p-\alpha \)-syn and HDL.

Our present study suggested that serum \( p-\alpha \)-syn reduction could be used as a biomarker for the early diagnosis of poststroke cognitive impairment. We also found that blood HDL levels were positively correlated with \( p-\alpha \)-syn levels and that abnormal HDL levels were associated with a high risk of cognitive impairment. In conclusion, we suggested that serum \( p-\alpha \)-syn was a biomarker for early warning of poststroke cognitive impairment and provided a basis for subsequent studies.

**Declarations**

**Funding**

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**Competing Interests**

The authors declare that they have no competing interests.

**Author Contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Yi Wang], [Yuning Li], [Yakun Gu], [Wei Ma] and [Yuying Guan]. The first draft of the manuscript was written by [Yuning Li]. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Data Availability**

The data that support the findings of this study are available from the corresponding author, [Jia Liu], upon reasonable request.

**Ethics approval**

This research project complied with the ethical review of the scientific research project of the Medical Ethics Committee of China Rehabilitation Research Center [Project Number: CRRC-IEC-EF-SC-005-01], and
all animal experiments were approved by the Animal Care and Use Committee of the Institute of Animal Management, Capital Medical University (Approval Number: AEEI-2021-058) and conducted in accordance with the ethical requirements.

**Consent to participate**

Informed consent was obtained from all individual participants included in the study.

**Consent to publish**

Not applicable.

**Acknowledgements**

Not applicable.

**References**


Figures
Figure 1

Accumulation of p-α-syn around the cerebral infarct area in dMCAO model mice. A: Representative images of laser speckles in an ischemic model. B: Cerebral blood flow (CBF) was quantified and expressed as percent change from baseline (pre-dMCAO). C-F: Changes in the behavior of ischemic animals at different time points. (C) Garcia score, (D) Grid-walking test, (E-F) Adhesive removal test (mean ± SEM, n = 15). G-H: Morphologic changes in the different coronal sections stained by MAP2 staining and...
visualized using a confocal laser scanning microscope, and quantification of the results. (G) Representative images of the different coronal sections of the ischemic model. (H) Statistical graph of the quantification of infarct volume data from (G). I: Representative images of MAP2 and p-α-syn immunofluorescence staining around the ischemic injury area (scale bar = 75 μm).

Figure 2
Accumulation of p-α-syn in the brain of hypoxic model mice. A: Schematic diagram of the mouse systemic hypoxia model. B-C: Changes in the behavior of hypoxia-induced animals at different time points. (B) Rotarod test, (C) Novel object recognition test (mean ± SEM, n = 10). D-E: Morphological changes in the cortex stained by Nissl staining and visualized using a light microscope, and quantification of the results. (D) Nissl staining of the cortex of the different groups (scale bar = 50 μm), (E) Statistical graph of the quantification of Nissl staining data from (D) (mean ± SEM, n = 3). F: Immunofluorescence staining of the cortex of the different hypoxic groups (scale bar = 20 μm). G: Representative single-cell stereogram from the Con and H14D in (F). H: Correlation statistics of p-α-syn and MAP2 fluorescence intensity in (F).
Figure 3

Serum p-α-syn level of patients with ischemic stroke (IS) is significantly reduced. A: p-α-syn level in the control and IS groups. B: Receiver operating characteristic (ROC) curve of p-α-syn for the diagnosis of stroke. The area under the ROC curve value was 0.7207 (95% CI: 0.6485–0.7929), *P<0.0001. C: The relationship between the p-α-syn level and time after IS (one-way ANOVA, mean ± SEM, *p<0.05, **p<0.005). D: The p-α-syn levels in the IS without diabetes group (diabetes−) and the IS with diabetes group (diabetes+). E: The p-α-syn levels in the IS without hyperlipidemia group (hyperlipidemia−) and the IS with hyperlipidemia group (hyperlipidemia+). F: The p-α-syn levels in the IS without hypertension group (hypertension−) and the IS with hypertension group (hypertension+). G: The p-α-syn levels in patients without stroke history (stroke history−) and patients with stroke history (stroke history+). H: The p-α-syn levels in different NIHSS score groups. I: The p-α-syn levels in the nonsmoking group (smoking−) and the smoking group (smoking+). J: The p-α-syn levels in the nondrinking group (drinking−) and the drinking group (drinking+). (D~E: Non-compared t-test, mean ± SD, *p<0.05, **p<0.01, ***p<0.005, ****p<0.0001)

Figure 4

Serum p-α-syn level could be a biomarker for diagnosing poststroke cognitive impairment. A: The p-α-syn levels in the control group, the ischemic stroke (IS) without cognitive impairment group (IS-none), and the IS with cognitive impairment group (IS-cog impairment). B: The p-α-syn levels in the different MMSE score groups. One-way ANOVA, mean ± SEM, *p<0.05, **p<0.01, ***p<0.005, ****p<0.0001. C: Receiver operating characteristic (ROC) curve of p-α-syn for the diagnosis of poststroke cognitive impairment. The area under the ROC curve value was 0.7660 (95% CI: 0.6798–0.8523), *P<0.0001.
**Figure 5**

**Correlation between biochemical indices and serum p-α-syn levels in patients with IS.** A Pearson’s correlation coefficient (r)-based heatmap for clinical indicators of the IS group. Blue, negative correlation; Red, positive correlation; *p<0.05, **p<0.01, ***p<0.005, ****p<0.0001.

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-1  -0.8  -0.6  -0.4  -0.2  0  0.2  0.4  0.6  0.8  1
Figure 6

Serum p-α-syn level was positively correlated with the HDL level in patients with IS. A: The p-α-syn levels in the control group, the IS patients without abnormal HDL level (IS-HDL(N)) group, and the IS patients with abnormal HDL level (IS-HDL(L)) group. B: Linear correlation of p-α-syn and HDL levels in all groups. C: Linear correlation of p-α-syn and HDL levels in the control group. D: Linear correlation of p-α-syn and
HDL levels in the IS group. E: Occurrence of cognitive impairment in the IS-HDL(N) group and the IS-HDL(L) group. *p<0.05, **p<0.01, ***p<0.005, ****p<0.0001.