Parkinsonism and Related Disorders 60 (2019) 105–110

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Alpha-synuclein in erythrocyte membrane of patients with multiple system atrophy: A pilot study

Genliang Liu[a,b,1], Chen Tian[c,d,e,f,1], Liyan Gao[g], Zhentang Cao[a,b], Tao Feng[a,b,h,*]

[a] Center for Neurodegenerative Disease, Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, 100050, China
[b] China National Clinical Research Center for Neurological Diseases, Beijing, 100050, China
[c] Department of Pathology, Peking University School of Basic Medical Sciences, Peking University, Beijing, 100191, China
[d] Department of Pathology, Peking University Third Hospital, Peking University, Beijing, 100191, China
[e] Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA
[f] Beijing Key Laboratory of Research and Transformation on Neurodegenerative Diseases Biomarkers, Beijing, 100191, China
[g] Department of Neurology, Qingdao Municipal Hospital, Qingdao University, Qingdao, 266071, China
[h] Parkinson's Disease Center, Beijing Institute for Brain Disorders, Capital Medical University, Beijing, 100069, China

ARTICLE INFO

Keywords:
Multiple system atrophy
α-synuclein
Erythrocyte membrane
Erythrocyte cytoplasm
Biomarker
SNCA
Electrochemiluminescence

ABSTRACT

Background: Multiple system atrophy(MSA) is a neurodegenerative disease characterized by intracellular α-synuclein deposits. There is an unmet need for blood-based biomarkers to diagnose MSA. Our previous studies have reported elevated α-synuclein levels in erythrocytes of MSA patients. However, α-synuclein protein in the membrane and cytoplasm of erythrocytes in MSA have not been investigated.

Methods: The membrane and cytoplasm were extracted from erythrocytes in 77 patients with MSA and 133 healthy controls. Levels of total and oligomeric α-synuclein were detected using Electrochemiluminescence assays. The correlations between α-synuclein levels and clinical characteristics were explored in MSA group. The diagnostic value of erythrocyte α-synuclein for MSA was determined by Receiver operator characteristic curve.

Results: α-synuclein levels in the erythrocyte membrane were significantly elevated in MSA patients compared with the healthy controls (total α-synuclein, $p = 0.003$; oligomeric α-synuclein/total α-synuclein, $p = 0.033$; oligomeric α-synuclein/protein, $p < 0.001$). The combination of total and oligomeric α-synuclein levels in erythrocyte membrane could efficiently distinguish MSA from healthy controls (sensitivity of 79.2%; specificity of 69.2%; area under the curve: 0.771). In contrast, no significant difference was found in erythrocyte cytoplasm α-synuclein levels. In the subgroup of 48 patients with probable MSA, there was a weakly negative correlation between oligomeric α-synuclein/protein in erythrocyte membrane and disease duration ($r = -0.336$; $p = 0.009$).

Conclusion: Our pilot study suggests that the membrane fraction of α-synuclein levels in erythrocyte were elevated in patients with MSA, and these levels may be decreased with the development of disease.

1. Introduction

Multiple system atrophy (MSA) is a progressive neurodegenerative disease that clinically presents with autonomic failure, parkinsonism and cerebellar ataxia [1]. MSA diagnosis is mainly based on clinical symptoms, with a high risk of misdiagnosis. The primary neuropathological feature of MSA is the presence of cytoplasmic inclusions mainly formed by α-synuclein (α-syn) protein in oligodendrocytes. α-syn is a presynaptic protein comprised of 140 amino acids and deposits at high levels in the brain. Moreover, oligomeric form of α-syn has been identified as the neurotoxic species in the synucleinopathies, such as MSA, Parkinson's Disease(PD) and dementia with Lewy bodies(DLB) [2]. Several studies have shown that levels of α-syn in cerebrospinal fluid (CSF) are associated with synucleinopathies, making them potential diagnostic biomarkers [3–6].

Besides the central nervous system(CNS), α-syn expression was also detected in blood cells [7]. Reports have revealed that plasma α-syn levels were elevated in patients with MSA and tended to decrease with the progression of the disease [8,9]. Although α-syn protein is abundant in red blood cells [7], erythrocyte α-syn levels in MSA have been rarely

* Corresponding author. Center for Neurodegenerative Disease, Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, China.
E-mail address: happyft@sina.com (T. Feng).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.parkreldis.2018.09.012
Received 9 May 2018; Received in revised form 5 September 2018; Accepted 10 September 2018

explored. Our previous study demonstrated that erythrocyte α-syn levels were higher in MSA patients than that in control groups [10]. Notably, α-syn was found to be deposited both in the cell membrane and cytoplasm of erythrocytes [11]. Since increasing evidence suggests that α-syn can specifically bind to the cell membrane [12,13], we therefore hypothesized that levels of α-syn in the cell membrane of erythrocytes may be altered in MSA, which may serve as a potential diagnostic marker.

In the present study, we aim to compare α-syn levels in the membrane and cytoplasm of erythrocytes between MSA and healthy control groups, respectively. Levels of total and oligomeric α-syn were measured by Electrochemiluminescence (ECL) assay, which is proposed to have high sensitivity and large dynamic range compared to classical ELISAs [14,15].

2. Patients and methods

2.1. Subjects

From January of 2015 to November of 2016, seventy-seven MSA patients (59 probable MSA and 18 possible MSA) and 133 healthy individuals without neurological diseases were recruited from the Department of Neurology in Beijing Tiantan Hospital, Capital Medical University. Patients were diagnosed by two experienced neurologists specialized in movement disorders and underwent an evaluation including medical history, physical and neurological examinations. The diagnosis of patients with MSA was based on the Second consensus criteria [16]. Exclusive criteria were: (1) secondary parkinsonism syndrome, corticobasal degeneration, progressive supranuclear palsy or spinocerebellar ataxia; (2) signs of cognitive impairment or dementia; (3) a history of blood diseases, stroke or brain tumor; (4) a family history of movement disorders. This study was approved by the Ethics Committee of Beijing Tiantan Hospital and was performed in accordance with the Declaration of Helsinki. Informed consent was obtained either from the participants or their closest relatives.

2.2. Erythrocyte collection and separation

Whole blood sample (5 ml) was collected in EDTA tubes and processed within 90 min after drawing. The blood was divided into three layers by centrifugation at 1500*g* for 10 min at 4 °C. The upper and middle layers were discarded to remove the plasma and the white blood cells. The cell pellet was washed three times with phosphate-buffered saline(PBS). The red blood cells were resuspended with aliquots (diluted 1:1 in ice-cold PBS) and stored at −80 °C. Samples were thawed only at the time of analysis.

To separate the cytosolic and membrane fractions, erythrocytes were subjected to two sequential freezes (−80 °C) and thaw (room temperature) cycles, then centrifuged at 14000 g for 10 min at 4 °C. The cytosolic protein-containing supernatant (cytosolic fraction) was removed and stored at −80 °C, while the membrane pellet was subsequently washed 3 times with PBS and centrifuged at 14000 g for 10 min at 4 °C. The membrane wash supernatant was discarded between washes. The extracted membrane pellet was then solubilized with STET lysis buffer (0.1 mmol/L NaCl, 10 mmol/L Tris pH8.0, 1 mmol/L EDTA, 1% Triton 100), incubated on ice for 30 min, and centrifuged at 14000 g for 10 min at 4 °C to pellet any remaining insoluble material. The membrane protein-containing supernatant (membrane fraction) was isolated and stored at −80 °C.

Protein concentration in erythrocyte membrane and cytosolic fractions was measured using the bicinchoninic acid (BCA) protein assay kit (Pierce, USA) at an absorbance of 562 nm relative to a protein standard.

2.3. Electrochemiluminescence immunoassays for total and oligomeric α-syn quantification

Standard proteins (Proteos, Inc.) were recombinantly phosphorylated or unphosphorylated α-syn monomers, or filaments. Filaments were purified by ion exchange chromatography, with purity assessed by SDS-PAGE, and concentration assessed by BCA protein assay as instructed by the manufacturer. Anti-α-syn MJFR-1 clone 12.1 (ab138501, Abcam), conformation-specific, anti-α-syn filaments MJFR-14 [17](ab209538, Abcam) were biotinylated and coated onto standard 96-well Meso Scale Discovery (MSD) U-Plex plates by incubating the plates with 1 μg/ml either MJFR-1 or MJFR-14 capture antibody solutions for 2 h at room temperature and under 600 rpm shaking, according to the manufacturer's instructions. After washing three times with 150 ml wash buffer (MSD), plates were blocked with 150 μL Diluent 35 (MSD) for 1 h while shaking at 600 rpm and at room temperature. After washing three times in wash buffer, samples and recombinant α-syn standards were incubated for 1 h at room temperature and while shaking at 600 rpm. After washing three times, Sulfo-TAG-labelled anti-α-synclein 42 antibody (1 μg/ml) was added followed by a 1 h room temperature incubation with 600 rpm shaking. After washing three times, 150 μL of 2 x Read Buffer T (MSD) was applied to each well and plates were analyzed in a Sector Imager 6000 (MSD). Data analysis was performed with the MSD Discovery Workbench 3.0 Data Analysis Toolbox. Due to individual variation of erythrocyte protein, the concentration of total α-syn in cell membrane was normalized to that of total protein in the cell membrane. Levels of oligomeric α-syn in cell membrane were presented for oligomeric α-syn per total α-syn in the cell membrane and oligomeric α-syn per protein in the cell membrane, respectively. Total α-syn and oligomeric α-syn in the cytoplasm were presented in a similar fashion.

2.4. Statistical analysis

Statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The *t*-test and the x^2 test were used to compare differences in clinical data and α-syn levels between groups. The Mann-Whitney test was used to compare differences between groups when the data were not normally distributed. Spearman correlation was used to correlate α-syn levels with participants' clinical characteristics. Binary logistic regression analysis and receiver operator characteristic (ROC) curve were used to determine the diagnostic value for each α-syn parameter separately and for combinations of parameters. The cutoff for the ROC analysis was determined using the Youden Index. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Demographic and clinical features

The demographic data and clinical characteristics of the MSA and healthy control groups are listed in Table 1. There was no difference in mean age and sex distribution between patients with MSA and healthy controls. According to the second consensus statement on MSA diagnosis [16], seventy-seven MSA patients were subdivided into two groups (56 patients with MSA-P and 21 patients with MSA-C). There were no significant differences in mean age, sex distribution, disease duration and H&Y score between MSA-P and MSA-C groups.

3.2. Comparison of α-syn between healthy controls and patients with MSA

In the erythrocyte membrane, both total and oligomeric α-syn levels were significantly higher in MSA group compared with that in the healthy control group (Table 1, Fig. 1). In addition, no differences in the cytoplasm α-syn levels were found between MSA and healthy control

Table 1
Demographic, clinical data and erythrocyte α-syn levels of the study population.

Group	MSA, n = 77	MSA-P, n = 56	MSA-C, n = 21	Controls, n = 133	*P*	
					MSA vs Controls	MSA-P vs MSA-C
Females/Males	27/50	20/36	7/14	65/68	0.052	0.845
Age	61.18 ± 7.38	61.93 ± 7.55	59.19 ± 6.68	59.85 ± 9.06	0.075	0.148
Age at onset	57.96 ± 7.01	58.54 ± 5.71	56.43 ± 5.71	NA	NA	0.242
Duration	3.22 ± 2.13	3.39 ± 4.07	2.76 ± 2.40	NA	NA	0.254
H&Y	2.701 ± 0.73	2.696 ± 0.74	2.714 ± 0.72	NA	NA	0.862
Erythrocyte membrane						
Total α-syn/protein, pg/ug	106.15 [78.08–130.61]	101.11 [76.24–127.21]	113.82 [84.28–301.18]	86.29 [70.71–110.79]	0.003*	0.217
Oligomeric α-syn/total α-syn, pg/ng	25.52 [16.55–30.88]	25.52 [18.33–29.92]	24.78 [9.26–36.51]	20.65 [14.49–27.36]	0.033*	0.945
Oligomeric α-syn/protein, pg/ug	2.487 [1.835–3.210]	2.224 [1.827–3.231]	2.856 [2.037–3.202]	1.692 [1.185–2.435]	< 0.001*	0.277
Erythrocyte cytoplasm						
Total α-syn/protein, pg/ug	77.86 [65.80–93.24]	86.02 [71.18–124.11]	75.71 [65.27–89.33]	76.55 [63.60–91.67]	0.435	0.107
Oligomeric α-syn/total α-syn, pg/ng	3.77 [2.86–4.57]	3.64 [2.53–4.17]	3.85 [3.07–4.61]	3.60 [2.69–4.86]	0.948	0.221
Oligomeric α-syn/protein, pg/ug	0.299 [0.249–0.350]	0.297 [0.246–0.356]	0.299 [0.255–0.345]	0.292 [0.233–0.365]	0.818	0.927

Abbreviation: MSA, multiple system atrophy; MSA-P, multiple system atrophy with predominant parkinsonism; MSA-C, multiple system atrophy with predominant cerebellar ataxia; NA, not applicable; H&Y, Hoehn & Yahr.
Data are represented as mean ± SD or median [25%–75%].
* This *P* value indicates a statistically significant difference.

groups (Table 1, Fig. 1).

3.3. Comparison of α-syn between MSA-C and MSA-P groups

In erythrocyte membrane and cytoplasm components, both total and oligomeric α-syn levels between MSA-P and MSA-C groups were not statistically different (Table 1).

3.4. Comparison of α-syn levels in the membrane and cytoplasm component of erythrocytes

Total and oligomeric α-syn levels were detected in the cell membrane and cytoplasm components in MSA and healthy control groups. In both MSA and healthy control groups, total and oligomeric α-syn levels in cell membrane were significantly higher than that in the cytoplasm.

3.5. Correlation of α-syn with age, disease duration and H&Y score in MSA

In the membrane and cytoplasm component of erythrocytes, total and oligomeric α-syn levels in patients with MSA were not correlated significantly with age, age at onset, disease duration or H&Y score. While in the subgroup of 59 patients with probable MSA, there was a weakly negative correlation between oligomeric α-syn/protein in the cell membrane and disease duration ($r = -0.336$; $p = 0.009$) (Fig. 2).

3.6. ROC curve analysis of α-syn in erythrocyte membrane between healthy controls and patients with MSA

Total α-syn discriminated MSA from healthy controls with a sensitivity of 94.5% and a specificity of 21.8% (cut-off > 67.49 pg/ug, AUC = 0.588). Oligomeric α-syn/protein and oligomeric α-syn/total α-syn discriminated MSA from healthy controls with a sensitivity of 80.5% and 71.1%, and a specificity of 53.4% and 60.2%, respectively (oligomeric α-syn/protein at a cut-off > 1.776 pg/ug, AUC = 0.696; oligomeric α-syn/total α-syn at a cut-off of > 22.14 pg/ng, AUC = 0.622). Binary logistic regression with Forword Likelihood Ratio method using total α-syn, oligomeric α-syn/total α-syn and oligomeric α-syn/protein levels from MSA patients and healthy controls, provided a model (−3.791 + 0.46*total α-syn + 1.432*oligomeric α-syn/protein-0.62*oligomeric α-syn/total α-syn) that significantly improved the ability to distinguish MSA from control with a sensitivity of 79.2% and specificity of 69.2% (AUC = 0.771) (Fig. 3).

4. Discussion

Our present study demonstrated that total and oligomeric α-syn levels of erythrocyte membrane were increased in MSA patients compared with healthy controls. The combination of total and oligomeric α-syn levels in the cell membrane yielded a sensitivity of 79.2% and a specificity of 69.2%, with an AUC of 0.771 for the differentiation between MSA patients and healthy controls. In contrast, α-syn levels in erythrocyte cytoplasm in MSA were not different from that of healthy controls.

Given the ease of sample collection, attempts have been made to diagnose MSA using blood-based biomarker. Substantial evidence indicates significant higher α-syn levels in plasma in MSA compared with healthy subjects [6,8,9]. However, α-syn levels of plasma could be artificially elevated due to contamination with erythrocytes or platelets during sample collection and processing [7,18]. Because RBCs contain more than 99% of α-syn levels in blood and detection of erythrocyte α-syn can avoid contamination arising from hemolysis [7], the present method should be more stable compared with detection of plasma.

To our knowledge, this is the first study evaluating total and oligomeric α-syn concentrations in erythrocyte membrane of patients with MSA. α-syn gene (SNCA) is specifically expressed in CNS and hematopoietic cells [7,15,19]. Several studies have examined erythrocyte α-syn levels in PD [20–22], which is regarded as one of the synucleinopathies. A previous study demonstrated that levels of oligomeric α-syn appeared higher in erythrocytes of PD patients as compared with controls [21]. Furthermore, Papagiannakis et al. [23] extracted the erythrocyte membrane components and found significantly increase in α-syn dimer levels of PD patients compared to healthy subjects. Our findings show that total and oligomeric α-syn levels deposited in erythrocyte membrane are also increased in MSA patients. However, the pathogenic mechanism of increased erythrocyte α-syn levels in synucleinopathies is still not fully understood. Recently, it has been shown that red blood cells and dopaminergic cells in the substantia nigra share similar regulatory factors that activate SNCA transcription [19], suggesting a parallel α-syn increase in erythrocytes and substantia nigra. Importantly, α-syn is a lipid-binding protein and interacts directly with cell membranes in physiological and pathological conditions [13]. Preclinical studies suggest that toxic species of α-syn, possibly dimers or oligomers, are formed based on lipid interactions [12,24]. Therefore, our results might also reflect the increased tendency of toxic species of α-syn being formed in the erythrocyte membrane of MSA. Moreover, several studies proved that oligomeric species of α-syn can disrupt cell membrane integrity and then lead to cellular dysfunction [12,13].

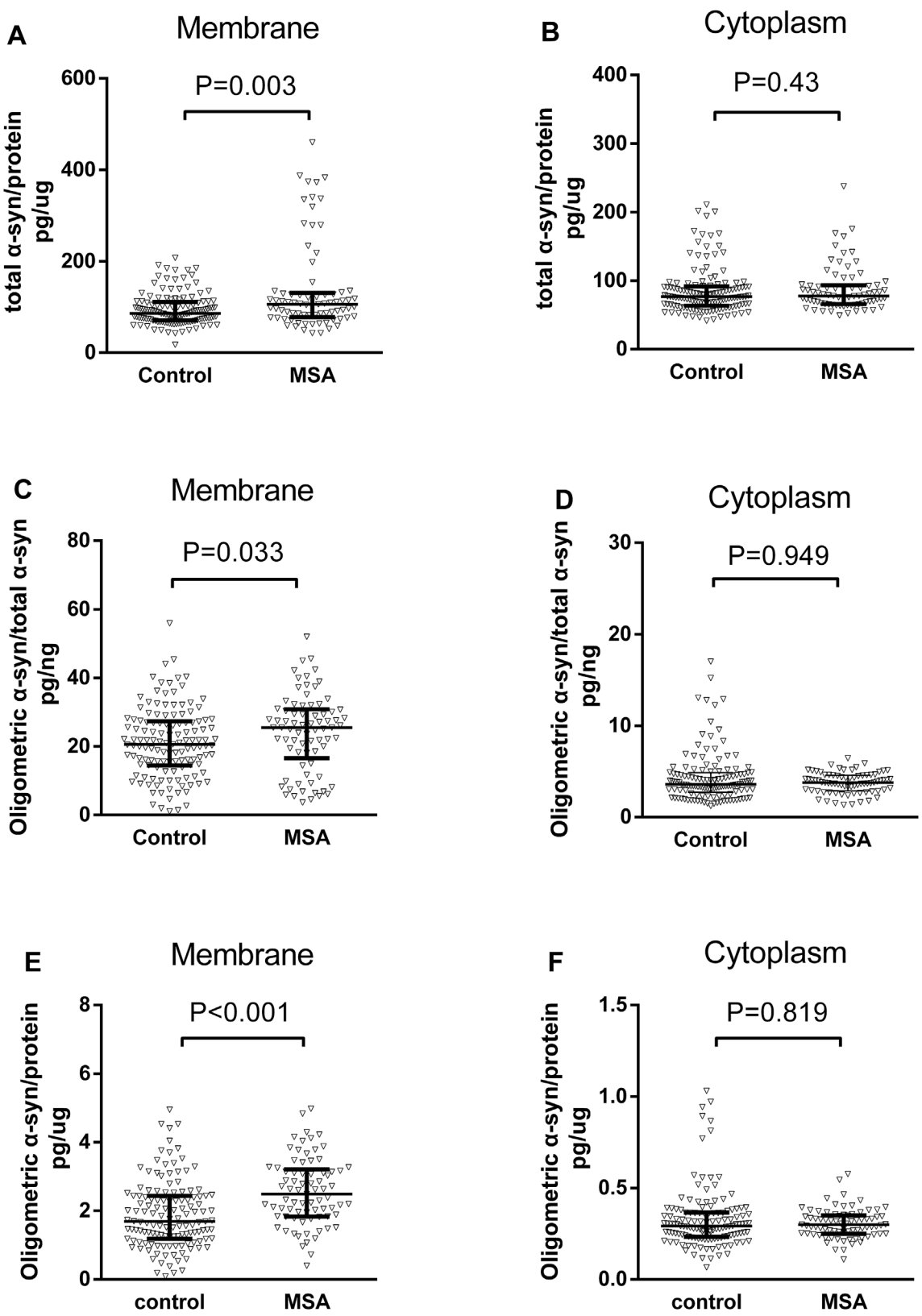

Fig. 1. Scatter plot of α-syn levels between HC and MSA groups. This scatter plot illustrates the median and interquartile range of α-syn levels. Long horizontal lines represent median values.

Further studies are needed to assess the specific function of α-syn and the morphology of erythrocyte membrane in MSA patients.

The α-syn levels in erythrocyte cytoplasm have not been previously investigated in synucleinopathies. In the present study, no significant difference in erythrocyte cytoplasm components was observed between MSA patients and healthy controls. Interestingly, we found total and oligomeric α-syn levels in cell membrane were significantly higher than that in the cytoplasm both in MSA patients and healthy controls.

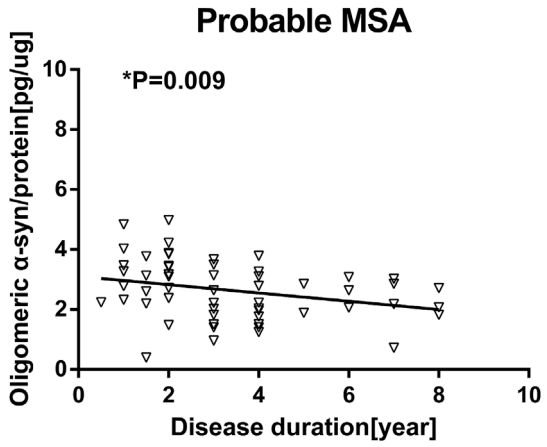

Fig. 2. Correlation between oligomeric α-syn/protein in erythrocyte membrane and disease duration in patients with probable MSA.

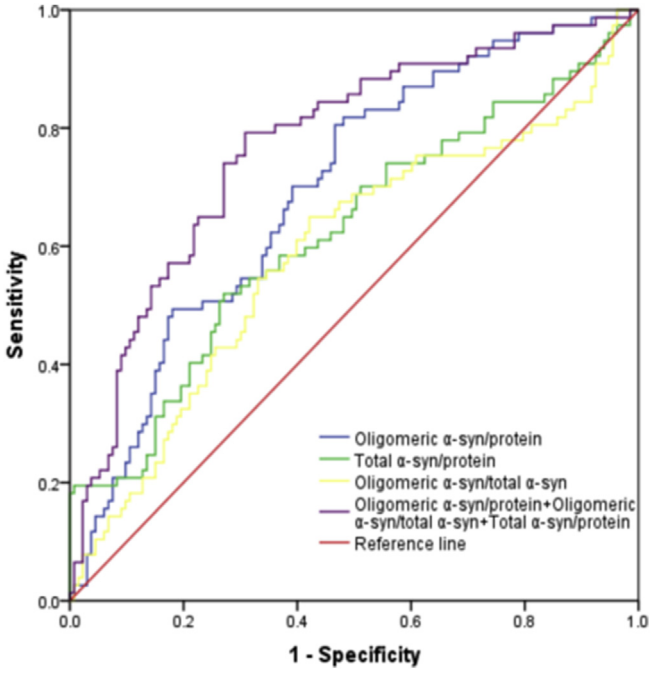

Fig. 3. ROC analysis of α-syn levels in erythrocyte membrane between healthy control and MSA groups. The yellow line is the ROC curve of oligomeric α-syn/total α-syn, and the AUC was0.588. The blue line is ROC curve of oligomeric α-syn/protein, and the AUC was 0.696. The green line is ROC curve of total α-syn, and the AUC was 0.622. The purple line is the ROC curve of the integrative model, and the AUC was 0.771.

Emerging evidence suggests that exosome-mediated transport could play a critical role in the processing of proteins involved in synucleinopathies [25,26]. Similar to one explanation for the elevated plasma levels of α-syn in patients with PD [26], it is hypothesized that α-syn is transported, at least in part, in association with membrane vesicles, whereas the majority of α-syn is accumulated in the membrane fraction of erythrocytes. It is important in future studies to access blood exosomes originated from CNS in MSA and explore their association with α-syn alterations in the brain.

Beyond these observations, our study showed α-syn oligomers/protein ratio in erythrocyte membrane negatively correlated with disease duration in the subgroup of patients with probable MSA. While erythrocyte α-syn levels were not correlated significantly with disease duration and age, age at onset, disease duration or H&Y score in both probable and possible MSA patients. The identification of biomarkers that reflect the disease progression could significantly improve future disease-modifying treatments. To date, blood-based α-syn levels were assessed with inconclusive results, one study found a correlation between plasma α-syn and disease severity, while another one did not [8,9]. This discrepancy may result from the differences in several variables, such as sample collection protocols and operating procedures [27]. Our findings should be confirmed by future larger and longitudinal studies.

There are some limitations to this study. Firstly, we did not access erythrocytes α-syn levels in patients with PD. Because of the relatively fast deterioration of clinical outcomes which could increase the accuracy of clinical diagnosis and allow monitoring disease modifying effects more rapidly, MSA is regarded as the most relevant clinical model for biomarker development as well as disease-modifying therapeutic studies in all synucleinopathies [28]. Further study should include patients with PD and DLB, in order to identify if this indicator can differentiate MSA from other synucleinopathies. Secondly, the potential confounding effect of dopaminergic medication has not been addressed. So far, the effect of dopaminergic drugs on the α-syn protein levels is controversial. It has been reported that specific dopaminergic medication does not change plasma α-syn levels [29]. Similarly, Mollenhauer et al. [6] did not record any significant effect of dopamine treatment on CSF and serum α-syn concentrations. Further study should investigate the effect of dopaminergic drugs to the erythrocyte α-syn levels.

In conclusion, we found that only the membrane-bound fraction of erythrocyte α-syn was elevated in patients with MSA, which would potentially serve as a diagnostic biomarker for MSA. Moreover, the specific elevation of α-syn in erythrocyte membrane might help understand the role of α-syn in MSA pathogenesis, and the potential correlation with disease duration would be useful in future disease-modifying trials. Large-scale and prospective studies are needed to validate our findings in patients with MSA and related synucleinopathies.

Acknowledgements

This research was supported by National Key R&D Program of China (No. 2016YFC1306501), National Natural Science Foundation of China (No. 81571226), Beijing Municipal Science and Technology Commission (No. Z17110700100000 and No. Z151100003915150).

References

[1] A. Fanciulli, G.K. Wenning, Multiple-system atrophy, N. Engl. J. Med. 372 (14) (2015) 1375–1376.
[2] Y.C. Wong, D. Krainc, alpha-synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies, Nat. Med. 23 (2) (2017) 1–13.
[3] T. Tokuda, S.A. Salem, D. Allsop, T. Mizuno, M. Nakagawa, M.M. Qureshi, J.J. Locascio, M.G. Schlossmacher, O.M. El-Agnaf, Decreased alpha-synuclein in cerebrospinal fluid of aged individuals and subjects with Parkinson's disease, Biochem. Biophys. Res. Commun. 349 (1) (2006) 162–166.
[4] M.J. Park, S.M. Cheon, H.R. Bae, S.H. Kim, J.W. Kim, Elevated levels of alpha-synuclein oligomer in the cerebrospinal fluid of drug-naive patients with Parkinson's disease, J. Clin. Neurol. 7 (4) (2011) 215–222.
[5] Z. Hong, M. Shi, K.A. Chung, J.F. Quinn, E.R. Peskind, D. Galasko, J. Jankovic, C.P. Zabetian, J.B. Leverenz, G. Baird, T.J. Montine, A.M. Hancock, H. Hwang, C. Pan, J. Bradner, U.J. Kang, P.H. Jensen, J. Zhang, DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease, Brain 133 (Pt 3) (2010) 713–726.
[6] B. Mollenhauer, J.J. Locascio, W. Schulz-Schaeffer, F. Sixel-Doring, C. Trenkwalder, M.G. Schlossmacher, alpha-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study, Lancet Neurol. 10 (3) (2011) 230–240.
[7] R. Barbour, K. Kling, J.P. Anderson, K. Banducci, T. Cole, L. Diep, M. Fox, J.M. Goldstein, F. Soriano, P. Seubert, T.J. Chilcote, Red blood cells are the major source of alpha-synuclein in blood, Neurodegener. Dis. 5 (2) (2008) 55–59.
[8] Z.F. Sun, X.S. Xiang, Z. Chen, L. Zhang, B.S. Tang, K. Xia, H. Jiang, Increase of the plasma alpha-synuclein levels in patients with multiple system atrophy, Mov. Disord. 29 (3) (2014) 375–379.
[9] P.H. Lee, G. Lee, H.J. Park, O.Y. Bang, I.S. Joo, K. Huh, The plasma alpha-synuclein levels in patients with Parkinson's disease and multiple system atrophy, J. Neural. Transm. 113 (10) (2006) 1435–1439.

[10] X. Wang, S. Yu, F. Li, T. Feng, Detection of alpha-synuclein oligomers in red blood cells as a potential biomarker of Parkinson's disease, Neurosci. Lett. 599 (2015) 115–119.
[11] M. Nakai, M. Fujita, M. Waragai, S. Sugama, J. Wei, H. Akatsu, C. Ohtaka-Maruyama, H. Okado, M. Hashimoto, Expression of alpha-synuclein, a presynaptic protein implicated in Parkinson's disease, in erythropoietic lineage, Biochem. Biophys. Res. Commun. 358 (1) (2007) 104–110.
[12] L. Tosatto, A.O. Andrighetti, N. Plotegher, V. Antonini, I. Tessari, L. Ricci, L. Bubacco, M. Dalla Serra, Alpha-synuclein pore forming activity upon membrane association, Biochim. Biophys. Acta 1818 (11) (2012) 2876–2883.
[13] P.K. Auluck, G. Caraveo, S. Lindquist, alpha-Synuclein: membrane interactions and toxicity in Parkinson's disease, Annu. Rev. Cell Dev. Biol. 26 (2010) 211–233.
[14] N. Kruse, W.J. Schulz-Schaeffer, M.G. Schlossmacher, B. Mollenhauer, Development of electrochemiluminescence-based singleplex and multiplex assays for the quantification of alpha-synuclein and other proteins in cerebrospinal fluid, Methods 56 (4) (2012) 514–518.
[15] A.H. Simonsen, B. Kuiperij, O.M. El-Agnaf, S. Engelborghs, S.K. Herukka, L. Parnetti, I. Rektorova, E. Vanmechelen, E. Kapaki, M. Verbeek, B. Mollenhauer, The utility of alpha-synuclein as biofluid marker in neurodegenerative diseases: a systematic review of the literature, Biomarkers Med. 10 (1) (2016) 19–34.
[16] S. Gilman, G.K. Wenning, P.A. Low, D.J. Brooks, C.J. Mathias, J.Q. Trojanowski, N.W. Wood, C. Colosimo, A. Durr, C.J. Fowler, H. Kaufmann, T. Klockgether, A. Lees, W. Poewe, N. Quinn, T. Revesz, D. Robertson, P. Sandroni, K. Seppi, M. Vidailhet, Second consensus statement on the diagnosis of multiple system atrophy, Neurology 71 (9) (2008) 670–676.
[17] L.B. Lassen, E. Gregersen, A.K. Isager, C. Betzer, R.H. Kofoed, P.H. Jensen, ELISA method to detect alpha-synuclein oligomers in cell and animal models, PLoS One 13 (4) (2018) e0196056.
[18] A. Atik, T. Stewart, J. Zhang, Alpha-synuclein as a biomarker for Parkinson's disease, Brain Pathol. 26 (3) (2016) 410–418.
[19] C.R. Scherzer, J.A. Grass, Z. Liao, I. Pepivani, B. Zheng, A.C. Eklund, P.A. Ney, J. Ng, M. McGoldrick, B. Mollenhauer, E.H. Bresnick, M.G. Schlossmacher, GATA transcription factors directly regulate the Parkinson's disease-linked gene alpha-synuclein, Proc. Natl. Acad. Sci. U. S. A. 105 (31) (2008) 10907–10912.
[20] S. Abd-Elhadi, A. Honig, D. Simhi-Haham, M. Schechter, E. Linetsky, T. Ben-Hur, R. Sharon, Total and proteinase K-resistant alpha-synuclein levels in erythrocytes, determined by their ability to bind phospholipids, associate with Parkinson's disease, Sci. Rep. 5 (2015) 11120.
[21] O.M. El-Agnaf, S.A. Salem, K.E. Paleologou, M.D. Curran, M.J. Gibson, J.A. Court, M.G. Schlossmacher, D. Allsop, Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease, Faseb. J. 20 (3) (2006) 419–425.
[22] H. Vicente Miranda, R. Cassio, L. Correia-Guedes, M.A. Gomes, A. Chegao, E. Miranda, T. Soares, M. Coelho, M.M. Rosa, J.J. Ferreira, T.F. Outeiro, Posttranslational modifications of blood-derived alpha-synuclein as biochemical markers for Parkinson's disease, Sci. Rep. 7 (1) (2017) 13713.
[23] N. Papagiannakis, C. Koros, M. Stamelou, A.M. Simitsi, M. Maniati, R. Antonelou, D. Papadimitriou, G. Dermentzaki, M. Moraitou, H. Michelakakis, L. Stefanis, Alpha-synuclein dimerization in erythrocytes of patients with genetic and non-genetic forms of Parkinson's Disease, Neurosci. Lett. (2017).
[24] R. Sharon, I. Bar-Joseph, M.P. Frosch, D.M. Walsh, J.A. Hamilton, D.J. Selkoe, The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease, Neuron 37 (4) (2003) 583–595.
[25] E. Emmanouilidou, K. Melachroinou, T. Roumeliotis, S.D. Garbis, M. Ntzouni, L.H. Margaritis, L. Stefanis, K. Vekrellis, Cell-produced alpha-synuclein is secreted in a calcium-dependent manner by exosomes and impacts neuronal survival, J. Neurosci. 30 (20) (2010) 6838–6851.
[26] M. Shi, C. Liu, T.J. Cook, K.M. Bullock, Y. Zhao, C. Ginghina, Y. Li, P. Aro, R. Dator, C. He, M.J. Hipp, C.P. Zabetian, E.R. Peskind, S.C. Hu, J.F. Quinn, D.R. Galasko, W.A. Banks, J. Zhang, Plasma exosomal alpha-synuclein is likely CNS-derived and increased in Parkinson's disease, Acta Neuropathol. 128 (5) (2014) 639–650.
[27] B. Laurens, R. Constantinescu, R. Freeman, A. Gerhard, K. Jellinger, A. Jeromin, F. Krismer, B. Mollenhauer, M.G. Schlossmacher, L.M. Shaw, M.M. Verbeek, G.K. Wenning, K. Winge, J. Zhang, W.G. Meissner, Fluid biomarkers in multiple system atrophy: a review of the MSA Biomarker Initiative, Neurobiol. Dis. 80 (2015) 29–41.
[28] P.O. Fernagut, B. Dehay, A. Maillard, E. Bezard, P. Perez, A. Pavy-Le Traon, O. Rascol, A. Foubert-Samier, F. Tison, W.G. Meissner, Multiple system atrophy: a prototypical synucleinopathy for disease-modifying therapeutic strategies, Neurobiol. Dis. 67 (2014) 133–139.
[29] R. Duran, F.J. Barrero, B. Morales, J.D. Luna, M. Ramirez, F. Vives, Plasma alpha-synuclein in patients with Parkinson's disease with and without treatment, Mov. Disord. 25 (4) (2010) 489–493.