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Gender differences in Parkinson's disease: focus on plasma alpha-synuclein

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Abstract Among promising biological markers proposed for Parkinson's disease (PD) and other disorders related to Lewy bodies, plasma alpha-synuclein assay has provided conflicting results mainly owing to the various laboratory assay techniques used and protein forms assayed. In this observational and exploratory cross-sectional study, using an immunoenzymatic technique, we assayed and compared total plasma alpha-synuclein concentrations in 69 patients with PD and 110 age-matched healthy control subjects. Two previously unreported findings concerned gender. First, plasma alpha-synuclein concentrations measured in the more advanced parkinsonian disease stages decreased in men, but not in women. Second, again only in men, plasma alpha-synuclein concentration was associated with cognitive impairments, hallucinations, and sleep disorders. These findings underline the gender-related differences in parkinsonian patients and indicate plasma alpha-synuclein expression as a potential biological marker for PD progression in men.

Keywords Parkinson's disease · Alpha-synuclein · Cognitive impairment · Biomarker

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Introduction

The major histological alteration responsible for Parkinson's disease (PD) is alpha-synuclein accumulation in the intra-cytoplasmic aggregates known as Lewy bodies. Increasing evidence suggesting that alpha-synuclein passes from the brain to blood (El-Agnaf et al. 2003; Lee et al. 2005; Dixon et al. 2005; Emmanouilidou et al. 2010; Silverberg et al. 2003; Tokuda et al. 2006; Hong et al. 2010) prompted studies to verify whether plasma alpha-synuclein assay reflects parkinsonian neurodegeneration. Studies in recent years investigating plasma alpha-synuclein as a possible biological marker for PD have provided conflicting results owing partly to the various assay techniques used, protein species assayed and small populations studied (Foulds et al. 2011; El-Agnaf et al. 2006; Duran et al. 2010; Li et al. 2007; Lee et al. 2006; Shi et al. 2010). Data are also lacking on whether alpha-synuclein plasma expression correlates with parkinsonian patients' clinical and epidemiological features. Nor have studies investigated possible gender differences in plasma alpha-synuclein concentrations in patients with PD. Having information on alpha-synuclein as a biomarker for PD would help diagnose PD, and monitor disease progression.

In this observational and exploratory cross-sectional study, using an immunoenzymatic technique, we assayed and compared total alpha-synuclein plasma concentrations in PD patients and age-matched healthy controls. Second,

we sought further information on possible associations and correlations between alpha-synuclein plasma expression and the major parkinsonian patients' clinical and epidemiological characteristics.

Subjects and methods

Subjects

The study included 69 patients with PD and 110 healthy age-matched control subjects, enrolled between January 2010 and July 2011 at the Neuromed Institute. All study procedures were approved by the institutional review board at the Neuromed Institute, conformed with the Declaration of Helsinki and all patients gave informed written consent. PD was diagnosed in accordance with the criteria of the United Kingdom Parkinson's Disease Society Brain Bank; patients were included consecutively, including those undergoing deep brain stimulation (DBS) or apomorphine pumps. Clinical assessment was based on the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS). The healthy control subjects were spouses of patients. They had no neurological diseases except for tension type headache. Among the 69 patients with PD enrolled, 26 had disease classified as predominantly hypokinetic, 30 had tremorigenic disease and 13 a mixed phenotype. Physical examination identified the left body side as involved by major slowing in 41 patients, tremor as the onset symptom in 40 patients and hypokinesia in 29 patients. Among the 25 patients with motor fluctuations, 23 subjects also had dyskinesias. Of the 69 patients enrolled ten had unilateral or bilateral microelectrodes implanted in the subthalamic nucleus for DBS. Clinical assessment showed that eight patients were receiving apomorphine alone, 18 patients levodopa alone, five patients dopamine agonists alone, 37 patients with levodopa plus dopamine agonists, and nine patients were receiving no medications. According to Hoehn and Yahr (HY) staging, 49 patients were in stage I and II and 20 in stage III. Six patients had familial PD. The mean age at disease onset was 53.86 ± 11.02 years and disease duration was 10.77 ± 7.30 years. The scores of MDS-UPDRS I, II, III and IV were 3.43 ± 3.49; 12.00 ± 7.94; 19.74 ± 12.36; 2.67 ± 3.72, respectively.

Laboratory assay

Venous blood from all patients was drawn in ethylenediaminetetraacetic acid (EDTA) tubes. Hemolyzed blood samples were discarded. Plasma samples were obtained by centrifugation at 2,500 rpm for 15 min, at 4 °C, aliquoted in 200 µl and stored at −80 °C until assay. Total plasma alpha-synuclein concentrations were determined by enzyme-linked immunosorbent assay (ELISA) based on alpha-synuclein capture antibody and alpha-synuclein antibody detection according to the manufacturer's protocol (Invitrogen Corporation, ref. KHB0061). Samples were analyzed in duplicate in blinded experiments. The optical density of the samples was determined at 450 nm with a Tecan Sunrise microplate reader (Tecan, Switzerland) and the results were expressed as ng alpha-synuclein/ml plasma.

Statistical analysis

Kolmogorov–Smirnov test was applied to test for a normal distribution of alpha-synuclein values. Parametric and nonparametric tests including the *t* test or Mann–Whitney test was used to compare means in independent samples when appropriate. Chi-square test was used for categorical variables. Spearman's correlation coefficient was obtained to investigate a possible relationship among clinical variables and alpha-synuclein values. To find out whether alpha-synuclein values differed according to patients' clinical variables, we also calculated the median value of age, age at onset, disease duration, MDS-UPDRS I, II, III and IV scores for the 69 patients and analyzed possible differences in the alpha-synuclein values between two subgroups with upper or lower median values. A logistic-regression model was run to evaluate the relation between upper or lower median alpha-synuclein values and the variables identified as significant or at borderline level in the univariate analysis. Odds ratios (ORs) with relative 95 % confidence intervals (CIs) were estimated. Values are expressed as mean ± standard deviation.

$p < 0.05$ were considered to indicate statistical significance. No statistical correction for multiple comparisons was conducted as this is an exploratory study. Data were analyzed for significance with a commercially available software package (SPSS, version 17.0).

Results

Kolmogorov–Smirnov test showed a statistically significant p value ($p = 0.023$) for the departure from normal distribution of alpha-synuclein values. For this reason, we have used nonparametric test with alpha-synuclein concentrations.

When blood samples were taken for total plasma alpha-synuclein assay, patients with PD and healthy control subjects were comparable for age and sex (Table 1). No significant differences were found in alpha-synuclein plasma concentrations in patients and healthy control subjects (Table 1). Concentrations tended to differ more widely in men than in women (men: 11.01 ± 4.33 vs.

Table 1 Comparison of age at evaluation, sex and plasma alpha-synuclein concentration in patients with Parkinson's disease and healthy controls

Age, sex and plasma alpha-synuclein (mean ± SD)	PD (mean ± SD)°	Healthy controls (mean ± SD)*	*p* value
Subjects	$n = 69$	$n = 110$	
Age (years)	64.59 ± 9.26	64.31 ± 9.17	n.s.
Sex (M/F)	40/29	57/53	n.s.
Alpha-synuclein (ng/ml)	10.55 ± 3.88	10.07 ± 3.16	n.s.
Men	$n = 40$	$n = 57$	
Age (years)	65.20 ± 9.08	64.88 ± 8.40	n.s.
Alpha-synuclein (ng/ml)	11.01 ± 4.33	9.79 ± 3.09	n.s.
Women	$n = 29$	$n = 53$	
Age (years)	63.76 ± 9.60	63.70 ± 9.99	n.s.
Alpha-synuclein (ng/ml)	9.90 ± 3.10	10.36 ± 3.24	n.s.

* No statistical difference was found between men and women health controls for age ($p = 0.50$) and alpha-synuclein ($p = 0.35$)

° No statistical difference was reported between men and women PD patients for age ($p = 0.53$) and alpha-synuclein ($p = 0.25$)

9.79 ± 3.09 ng/ml, $p = 0.11$; women: 9.9 ± 3.1 vs. 10.36 ± 3.24 ng/ml, $p = 0.54$) (Table 1).

Nor were significant correlations found between alpha-synuclein plasma concentrations and age in the 179 participants overall ($r = 0.133$; $p = 0.08$), in the 69 patients ($r = 0.195$; $p = 0.11$) or in the healthy control group ($r = 0.098$; $p = 0.31$).

No significant associations were found between plasma alpha-synuclein concentrations and familiarity for PD, symptoms at onset, predominantly affected body side, clinical phenotype, HY stage, oral dopaminergic therapy, subcutaneous apomorphine infusion, unilateral or bilateral DBS, dyskinesias, or motor fluctuations in the 69 patients (data not shown). Nor were correlations found between plasma alpha-synuclein concentrations and scores on the MDS-UPDRS sections I, II, III, IV, age at onset or disease duration in the 69 patients (data not shown).

The median value of age, age at onset, disease duration, MDS-UPDRS I, II, III and IV scores for the 69 patients were 66, 57, 10, 3, 11, 17 and 0, respectively (Table 2). Statistically significant differences in the alpha-synuclein values between two subgroups with upper or lower median values in the age ($p = 0.02$) and age at onset ($p = 0.01$) were found for 69 patients (Table 2). In these analyses, the alpha-synuclein values increase with age (Table 2).

Conversely, plasma alpha-synuclein concentrations differed according to PD stage and gender. In men, they were lower at HY scale stage III than at stages I and II (8.09 ± 0.82 vs. 11.63 ± 4.52 ng/ml; $p = 0.05$), but in women were similar at all stages (10.46 ± 2.66 vs. 9.46 ± 3.44 ng/ml; $p = 0.39$) (Table 2).

No gender-related differences were found between alpha-synuclein levels and the other clinical characteristics investigated, such as unilateral or bilateral DBS, oral dopaminergic therapy, subcutaneous apomorphine infusion, clinical phenotype, symptoms at onset, familiarity, and predominantly affected body side (data not shown). Finally, we found a significant inverse correlation between alpha-synuclein levels and MDS-UPDRS IV scores in men but not in women (men: $r = -0.33$, $p = 0.04$; women: $r = 0.08$, $p = 0.67$).

In men, we found a statistically significant differences in the alpha-synuclein values between two subgroups with upper or lower median values in the age ($p = 0.04$), age at onset ($p = 0.02$) and disease duration ($p = 0.05$) (Table 2). Lower mean alpha-synuclein concentrations were associated with a longer disease duration (Table 2). None statistically significant difference was found in women (Table 2).

In the 69 patients with PD overall, no significant association appeared between alpha-synuclein levels and characteristics detected in MDS-UPDRS scores for section I, whereas in patients subgrouped by gender alpha-synuclein concentrations were significantly lower in men with cognitive disorders than in those without (8.78 ± 2.51 vs. 12.09 ± 4.64 ng/ml, $p = 0.02$) (Table 3). Similarly, alpha-synuclein values were significantly lower in men with hallucinations, psychosis and apathy than in those without (7.81 ± 1.89 vs. 11.69 ± 4.41 ng/ml, $p = 0.03$; and 8.16 ± 3.01 vs. 11.62 ± 4.36 ng/ml, $p = 0.05$), whereas no differences were found in women ($p = 0.51$; and $p = 0.93$) (Table 3). Plasma alpha-synuclein concentrations were significantly higher in men reporting sleep disorders than in those without (12.15 ± 9.20 vs. 10.81 ± 3.01 ng/ml, $p = 0.05$), but no differences were found in women ($p = 0.79$) (Table 3). Nor were gender-related differences found in the clinical characteristics depression, pain and constipation.

Finally, a regression logistic model showed that the presence of cognitive deficit was associated in men with a lower median value of alpha-synuclein concentrations (≤11.08 ng/ml) adjusted for age, age at onset and disease duration (OR = 0.144; 95 % CI 0.023–0.895) (Table 4). The other clinical variables identified as significant or at borderline level in the univariate analysis in men (hallucinations and psychosis, apathy and sleep disorders) (Table 3) were not significant when included in the regression logistic model.

Discussion

In this observational and exploratory cross-sectional study, we have not observed any significant difference between

Table 2 Plasma alpha-synuclein concentrations (mean ± SD ng/ml) in the 69 patients with Parkinson's disease according to median clinical variables values by sex

Clinical characteristics	Men ($n = 40$)	p value	Women ($n = 29$)	p value	All patients ($n = 69$)	p value
Age (years)						
≤66	9.62 ± 3.38	0.04	9.25 ± 3.20	n.s.	9.46 ± 3.26	0.02
>66	12.40 ± 4.79		10.61 ± 2.95		11.67 ± 4.18	
Age at onset (years)						
≤57	9.34 ± 3.25	0.02	9.71 ± 3.04	n.s.	9.53 ± 3.10	0.01
>57	12.38 ± 4.68		10.36 ± 3.38		11.79 ± 4.39	
Disease duration (years)						
≤10	12.02 ± 4.85	0.05	9.56 ± 3.06	n.s.	11.22 ± 4.46	n.s.
>10	9.32 ± 2.66		10.16 ± 3.20		9.77 ± 2.94	
Hoehn–Yahr stage						
I–II	11.63 ± 4.52	0.05	9.46 ± 3.44	n.s.	10.92 ± 4.29	n.s.
III	8.09 ± 0.82		10.46 ± 2.66		9.63 ± 2.46	
MDS-UPDRS I (scores)						
≤3	10.83 ± 2.94	n.s	9.33 ± 3.18	n.s.	10.17 ± 3.11	n.s.
>3	11.28 ± 5.94		11.00 ± 2.78		11.17 ± 4.90	
MDS-UPDRS II (scores)						
≤11	10.98 ± 3.28	n.s.	9.87 ± 3.29	n.s.	10.51 ± 3.28	n.s.
>11	11.05 ± 5.35		9.95 ± 3.01		10.58 ± 4.48	
MDS-UPDRS III (scores)						
≤17	11.32 ± 2.56	n.s.	9.56 ± 3.53	n.s.	10.77 ± 2.96	n.s.
>17	10.55 ± 6.19		10.12 ± 2.90		10.32 ± 4.67	
MDS-UPDRS IV (scores)						
≤0	11.88 ± 4.77	n.s.	9.77 ± 3.07	n.s.	11.14 ± 4.33	n.s.
>0	9.40 ± 2.86		10.03 ± 3.23		9.73 ± 3.02	

n.s. not significant

the PD patients and the healthy controls in plasma alpha-synuclein concentrations measured using an immunoenzymatic technique. No association or correlation has been found between clinical variables and the plasma alpha-synuclein concentrations.

Unexpectedly, a statistical stratified by sex analysis showed some differences between men and women PD patients.

In men, alpha-synuclein concentrations differed significantly according to disease progression: they were lower in HY stage III than in stages I and II and in disease duration >10 years. The second previously unreported finding is the significant association between plasma alpha-synuclein concentrations and cognitive impairment, hallucinations, psychosis, apathy, sleep disturbances in men but not in women. However, in the multivariate analysis only cognitive impairment showed a significant association with lower alpha-synuclein concentrations.

We briefly can speculate on the possible reasons for this gender difference in PD patients.

An intriguing explanation for decreased alpha-synuclein concentrations in men but not in women as PD progresses is that intracellular alpha-synuclein aggregation differs in men and women owing to a protective hormonal effect in women. Estrogens, the hormones that especially influence women, dose-dependently inhibit alpha-synuclein aggregation (Hirohata et al. 2009). In particular, estriol and estradiol destabilize preformed fibrillar alpha-synuclein in vitro (Hirohata et al. 2009). Several studies indicate that alpha-synuclein is physiologically secreted in the extracellular spaces then reaches the plasma (El-Agnaf et al. 2003; Lee et al. 2005; Emmanouilidou et al. 2010). It could do so by passing directly through the blood–brain barrier, which is structurally altered in neurodegenerative diseases, or by draining into the cerebrospinal fluid and then into the bloodstream. If intracellular alpha-synuclein aggregation impairs protein secretion in the extracellular spaces and consequently its passage into the bloodstream (Li et al. 2007), or if protein secretion decreases owing to cell death triggered by protein aggregation then the decreased plasma alpha-synuclein concentration we reported in men as PD progressed may reflect greater intracellular accumulation in men than in women. Some studies have nevertheless shown that alpha-synuclein secretion increases during cellular

Table 3 Plasma alpha-synuclein concentrations in patients with Parkinson's disease for subitems part I Movement Disorders Society-Unified Parkinson's disease rating scale by sex

MDS-UPDRS part I subitems	Men ($n = 40$)	p value	Women ($n = 29$)	p value	All ($n = 69$)	p value
Cognitive impairment						
Yes	8.78 ± 2.51 (13)[a]	0.02	10.27 ± 2.97 (13)	n.s.	9.53 ± 2.80 (26)	n.s.
No	12.09 ± 4.64 (27)		9.61 ± 3.27 (16)		11.17 ± 4.31 (43)	
Hallucinations and psychosis						
Yes	7.81 ± 1.89 (7)	0.03	10.48 ± 3.15 (9)	n.s.	9.31 ± 2.94 (16)	n.s.
No	11.69 ± 4.41 (33)		9.65 ± 3.13 (20)		10.92 ± 4.07 (53)	
Mood and depression						
Yes	11.00 ± 5.38 (21)	n.s.	10.50 ± 2.96 (20)	n.s.	10.76 ± 4.32 (41)	n.s.
No	11.03 ± 2.91 (19)		8.59 ± 3.19 (9)		10.25 ± 3.16 (28)	
Anxiety						
Yes	1.44 ± 6.51 (12)	n.s.	0.82 ± 3.17 (5)	n.s.	11.12 ± 5.63 (17)	n.s.
No	10.83 ± 3.12 (28)		10.35 ± 3.07 (24)		10.36 ± 3.15 (52)	
Apathy						
Yes	8.16 ± 3.01 (7)	0.05	10.05 ± 3.41 (4)	n.s.	8.85 ± 3.14 (11)	n.s.
No	11.62 ± 4.36 (33)		9.89 ± 3.13 (25)		10.87 ± 3.94 (58)	
Sleep disorders						
Yes	12.15 ± 9.20 (6)	0.05	13.31 ± 2.84 (4)	n.s.	11.41 ± 7.11 (10)	n.s.
No	10.81 ± 3.01 (34)		9.85 ± 3.20 (25)		10.40 ± 3.10 (59)	
Daytime sleep						
Yes	11.81 ± 6.49 (13)	n.s.	11.18 ± 2.15 (2)	n.s.	11.72 ± 6.04 (15)	n.s.
No	10.63 ± 287 (27)		9.81 ± 3.17 (27)		10.22 ± 3.02 (54)	
Pain						
Yes	13.87 ± 9.23 (5)	n.s.	9.68 ± 2.83 (5)	n.s.	11.77 ± 6.80 (10)	n.s.
No	10.60 ± 3.18 (35)		9.96 ± 3.21 (24)		10.34 ± 3.18 (59)	
Urinary disturbances						
Yes	9.82 ± 2.94 (7)	n.s.	10.98 ± 1.87 (2)	n.s.	10.08 ± 2.68 (9)	n.s.
No	11.26 ± 4.57 (33)		9.83 ± 3.19 (27)		10.62 ± 4.04 (60)	
Constipation						
Yes	12.43 ± 6.86 (11)	n.s.	9.53 ± 2.52 (4)	n.s.	11.66 ± 6.06 (15)	n.s.
No	10.47 ± 2.86 (29)		9.97 ± 3.23 (25)		10.24 ± 3.02 (54)	
Fatigue						
Yes	12.92 ± 6.44 (11)	n.s.	10.31 ± 2.84 (4)	n.s.	12.22 ± 5.72 (15)	n.s.
No	10.29 ± 3.06 (29)		9.85 ± 3.19 (25)		10.08 ± 3.10 (54)	

Presence of disturbance (scores 1–4): yes; absence of disturbance: no

[a] number of patients

Table 4 Logistic Regression Model on relation between alpha-synuclein values (subdivided upper and lower median value of ≤11.08 ng/ml) and cognitive status in male PD patients

	B	E.S.	Wald	*df*	Sig.	OR	95 % CI	
							Lower	Upper
Age (>66 vs ≤66 years)	1.475	0.973	2.298	1	0.130	4.370	0.649	29.412
Disease duration (>10 vs ≤10 years)	−1.369	0.968	1.998	1	0.157	0.254	0.038	1.697
Age at onset (>57 vs ≤57 years)	−0.026	0.939	0.001	1	0.978	0.975	0.155	6.135
Cognitive status (altered vs normal)	−1.935	0.931	4.324	1	0.038	0.144	0.023	0.895
Constant	0.226	0.683	0.109	1	0.741	1.253		

stress (Jang et al. 2010); extracellular alpha-synuclein protein secretion might therefore increase as a cellular defense mechanism aimed at removing pathological proteins. Whether the low plasma alpha-synuclein concentrations we observed in men with advanced PD (HY stage III) reflect the estrogen-induced anti-aggregant effects on alpha-synuclein fibrils prompts further study.

Another possible reason why alpha-synuclein concentrations decreased in men as PD progressed is a gender-related difference in quantitative alpha-synuclein expression in the central nervous system, possibly related to hormonal factors. Sex hormones may alter alpha-synuclein expression in the brain modulating estrogen receptors (Marwarha et al. 2011). Studying the gender-associated genetic expression in dopaminergic neurons from post-mortem brains, Cantuti-Castelvetri et al. (2007) observed a female-associated gene up-regulation in signal transduction and neuronal maturation. They also observed a men-associated up regulation of genes directly involved in PD pathogenesis, including alpha-synuclein. Men therefore seem to produce more central nervous system alpha-synuclein than women. Conversely, Simunovic et al. (2010) showed alpha-synuclein gene downregulation in male dopamine neurons in sporadic PD. These preliminary data suggest that alpha-synuclein gene expression might differ in males and females.

Alternatively, instead of reflecting the neurodegenerative process the gender-related differences in plasma alpha-synuclein levels could reflect a sex-related difference in alpha-synuclein expression in peripheral blood cells. Alpha-synuclein is also present in all blood cells, especially in platelets (Barbour et al. 2008). Hence, we cannot exclude the possibility that plasma alpha-synuclein may derive from blood cell secretion, even though no current data show whether blood cells secrete alpha-synuclein. Several studies on peripheral alpha-synuclein examined qualitative or quantitative protein expression and found no differences between males and females or between patients and healthy control subjects (Tan et al. 2005; Michell et al. 2005; Brighina et al. 2010).

Precisely what the lower plasma alpha-synuclein concentrations in men with clinical MDS-UPDRS I scores showing cognitive impairment and apathy than in those without reflect remains open to question. Cognitive deficits and apathy in Parkinson's disease may correlate with more widespread neurodegeneration involving the cerebral cortex and in particular the fronto-basal-ganglia circuits starting from the dorsolateral prefrontal cortex and the cingulate gyrus, as well as with multiple cholinergic, noradrenergic and serotonergic neurotransmitter abnormalities (Ferrer 2011). The observed differences in plasma alpha-synuclein concentrations between men and women patients with PD may therefore reflect different gender-related protein expression patterns. The hallucinations and psychosis in our male patients were associated with lower alpha-synuclein levels: even though these symptoms may be an adverse effect induced by dopaminergic therapy, Duran et al. (2010) showed that dopaminergic therapy leaves plasma alpha-synuclein values unchanged.

In men, but not in women, alpha-synuclein values differed also according to another clinical characteristic, sleep disorders, and were significantly higher in male patients with sleep disorders than in those without. The increased plasma alpha-synuclein concentrations we observed only in male patients could reflect the male prevalence in these disorders: in example, several studies have shown an association between REM sleep behavior disorders and male PD patients (Cantuti-Castelvetri et al. 2007; Yoritaka et al. 2009; Scaglione et al. 2005).

Finally, the absence of differences in plasma alpha-synuclein concentrations between patients and controls partially contrasts with results from comparable studies using a similar immunoenzymatic assay (Duran et al. 2010; Lee et al. 2006; Park et al. 2011; Mollenhauer et al. 2011). This could be principally due to different clinical stage of PD patients, the inclusion of familiar PD patients and type of controls.

None of the previous studies analyzed data for gender (Duran et al. 2010; Lee et al. 2006; Park et al. 2011; Mollenhauer et al. 2011).

Although no differences in plasma alpha-synuclein levels emerged between patients and controls, it cannot be entirely ruled out the possibility that the alpha-synuclein could also represent a marker of disease in men, since the difference in measured concentration between PD males and health controls males ($p = 0.11$) (Table 1) may become significant increasing the number of subjects (a false negative result).

A limitation of our study is that we do not have used specific scales for nonmotor symptoms of Parkinson's disease. Also in our 10 ten familial cases we have not performed any genetic test on monogenic PD forms that may have debatable synuclein pathology (Parkin, LRRK2, GBA). Moreover, we have not used a duplicate test using another ELISA to confirm or not the findings.

The main strengths in this study are in the assessment between alpha-synuclein concentrations and many clinical variables and in the stratified analysis by sex.

In conclusion, our findings suggest that alpha-synuclein, as assessed by enzyme immunoassay designed to detect total soluble forms, is a marker of PD progression only in men, and is associated with cognitive disorders, hallucinations, psychosis, and sleep disorders. The possible gender-related role of plasma alpha-synuclein in patients with PD merits confirmation in a larger population.

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Conflict of interest None.

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