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# Cerebrospinal Fluid α-Synuclein Predicts Cognitive Decline in Parkinson Disease Progression in the DATATOP Cohort

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Most patients with Parkinson disease (PD) develop both cognitive and motor impairment, and biomarkers for progression are urgently needed. Although α-synuclein is altered in cerebrospinal fluid of patients with PD, it is not known whether it predicts motor or cognitive deterioration. We examined clinical data and α-synuclein in >300 unmedicated patients with PD who participated in the deprenyl and tocopherol antioxidative therapy of parkinsonism (DATATOP) study, with up to 8 years of follow-up. Longitudinal measures of motor and cognitive function were studied before (phase 1) and during (phase 2) levodopa therapy; cerebrospinal fluid was collected at the beginning of each phase. Correlations and linear mixed models were used to assess α-synuclein association with disease severity and prediction of progression in the subsequent follow-up period. Despite decreasing α-synuclein (phase 1 to phase 2 change of $-0.05 \pm 0.21$ log-transformed values, $P < 0.001$), no correlations were observed between α-synuclein and motor symptoms. Longitudinally, lower α-synuclein predicted better preservation of cognitive function by several measures [Selective Reminding Test total recall α-synuclein × time interaction effect coefficient, −0.12 ($P = 0.037$); delayed recall, −0.05 ($P = 0.002$); New Dot Test, −0.03 ($P = 0.002$)]. Thus, α-synuclein, although not clinically useful for motor progression, might predict cognitive decline, and future longitudinal studies should include this outcome for further validation. *(Am J Pathol 2014, 184: 966–975; http://dx.doi.org/10.1016/j.ajpath.2013.12.007)*

In addition to disabling motor symptoms that become more severe over time, it is increasingly recognized that Parkinson disease (PD) progression also includes development of significant nonmotor symptoms. Of particular concern is cognitive decline as the disease progresses, with most patients eventually developing dementia,[1,2] with devastating consequences for both patients and caregivers. However, the natural course of motor and cognitive decline in PD can vary substantially, with individual patients exhibiting slower decline or precipitous decreases in motor or cognitive function, likely depending on variation in the underlying pathological characteristics. There is no method to identify patients at risk of fast decline, and, because the mechanisms by which it occurs are not understood, no treatments exist to alter the course of the process.

α-Synuclein (α-syn) is the primary component of the Lewy bodies that are diagnostic of PD, and has been implicated in the pathogenesis of PD by much evidence, including the existence of early-onset familial forms caused by mutations in its gene and consistent association with sporadic PD in genome-wide association studies.[3–5] Although mechanisms remain to be investigated, α-syn protein has also been shown in several large studies to be lower in the cerebrospinal fluid (CSF) of patients with PD and related synucleinopathies

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(eg, Lewy body dementia and multiple system atrophy), compared with controls.[6–10] CSF α-syn does not typically correlate with severity of motor impairment in studies with large cohorts[6,8,11]; however, these were generally performed in cross-sectional cohorts, often confounded by the exposure of all subjects to dopamine therapy. Human studies to assess the role of α-syn in cognitive decline or dementia are largely an uncharted territory.

The deprenyl and tocopherol antioxidative therapy of parkinsonism (DATATOP) study remains the largest cohort assembled, with longitudinal collection of biological fluid and clinical data of patients with PD.[12,13] DATATOP subjects were recruited at early disease stages, without apparent signs of dementia and before needing dopamine-supplementing drugs, and were extensively characterized by longitudinal clinical assessment, including measures of motor and cognitive function, making this cohort ideal for studying PD progression. In addition, each subject contributed CSF samples at two time points, allowing investigation of the question of whether biomarkers at unmedicated baseline or the beginning of levodopa therapy can predict motor or cognitive progression.

This study examined the relationships between CSF α-syn and measures of PD severity and progression. The longitudinal alterations in CSF α-syn during unmedicated PD progression were determined. Cross-sectional correlations of CSF α-syn with motor and cognitive measures, at early disease stages and after significant PD progression, were assessed. Finally, it was determined whether CSF α-syn in early unmedicated PD, or just at the point at which medication becomes necessary, predicts motor or cognitive progression in the subsequent time period.

## Materials and Methods

### Subjects and Clinical Measures

Procedures were approved by all institutions participating in the study, and written consent was obtained from all subjects under the supervision of institutional review boards of the study sites.

All subjects were participants in the DATATOP study, a placebo-controlled, double-blind study to determine the effectiveness of the monoamine oxidase type B inhibitor, deprenyl, and the antioxidant, α-tocopherol, in delaying PD progression. A total of 800 subjects with early, unmedicated PD were recruited between September 1987 and November 1988. Subjects had mild PD symptoms not initially requiring dopamine replacement, and did not meet the study criteria for dementia [Mini-Mental Status Examination (MMSE) score, <23]. Subjects were randomly assigned to one of four treatment groups: placebo, deprenyl, α-tocopherol, or both deprenyl and α-tocopherol. The primary end point was defined as the time at which a clinician (blind to the study treatment group) determined that the subject's PD motor symptoms had progressed to the point of requiring levodopa therapy. Many of the subjects who reached end point before completion of their 24-month enrollment period were restarted in blinded manner on their previously assigned study drugs. After subjects had been followed up for an average of 14 months, the study was discontinued because of the observation that deprenyl had positive effects on progression of PD motor symptoms, and all subjects were transitioned to open-label administration of deprenyl for approximately 18 months.

Cognitive performance and other clinical measurements [including the Unified Parkinson Disease Rating Scale (UPDRS), MMSE, and Hoehn and Yahr scales] were assessed at baseline, and then every 6 months afterward, continuing after end point, for up to 6.9 years (average follow-up, 1.8 years). Longitudinal data are separated into phase 1, consisting of the time period beginning at study entry until end point, and phase 2, beginning at the initiation of levodopa therapy and continuing until the end of follow-up. CSF samples were collected at entry into the study (beginning of phase 1) and at the time of end point (beginning of phase 2).

Previous studies did not detect differences in cognitive decline between treatment groups[14]; therefore, all treatment groups are included in this study. Because the current study focused on changes in CSF biomarker levels, and cognitive decline, for which changes caused by disease progression are likely to be slow, 110 subjects with <6 months of follow-up were excluded, as in a previous investigation.[15] Also excluded were those who withdrew after 6 months (34 subjects), whose initial PD diagnoses were found to be incorrect (45 subjects), and any subjects with missing UPDRS or CSF data at the beginning of phase 1 ($n = 63$) or phase 2 ($n = 189$). The remaining 403 subjects were included in the current analyses that examined data from phase 1. Of these subjects, a total of 305 reached their end points by the end of a deprenyl open-label trial and continued after starting levodopa, and were thus included in phase 2 analyses.

### Cognitive Measures

The DATATOP study included several measures of cognitive performance. Only those for which longitudinal data were available through the end of the follow-up period were included in this study. These tests were as follows: total and delayed recall selective reminding tests[16] [Selective Reminding Test (SRT)-Total and SRT-Delayed, respectively; measures of verbal learning and memory], Symbol Digit Modalities Test (SDMT[17]; a test of visuospatial working memory/processing speed), and New Dot Test (visuospatial working memory).

### *APOE* Genotype

Genomic DNA was available from 199 DATATOP participants who participated in phase 2 analyses. *APOE* genotyping was performed by using a matrix-assisted laser desorption/

ionization time-of-flight mass spectrometry technology combined with the homogeneous mass-extend reaction, as previously described.[18]

### CSF Assays

The procedure for CSF collection has been described elsewhere.[19] Briefly, lumbar puncture was performed between 6 and 10 AM, and samples were collected in measured aliquots, which were immediately placed on ice until freezing at −70°C. All sites used the same collection procedures. CSF samples remained frozen until immediately before measurement of α-syn levels by Luminex assays (Luminex, Austin, TX), according to our previously published protocol.[6] All samples were evaluated using a LiquiChip Luminex 200 Workstation (Qiagen, Hilden, Germany). Hemoglobin (Hgb) was measured in all samples by ELISA (Bethyl Lab, Inc., Montgomery, TX), according to the manufacturer's instructions. On the basis of our previous findings that α-syn correlates strongly with Hgb in samples with Hgb >200 ng/mL,[6] samples exceeding this cutoff were excluded from the analysis. Although the CSF samples used in this study were archived samples (many were stored frozen for >20 years), α-syn values were consistent with those from more recently collected CSF measured by the same method in previous studies.[6]

### Analysis

Because subjects began levodopa therapy after the end of phase 1, UPDRS scores (which are expected to be markedly affected by drug treatment) are difficult to interpret through this transition. Furthermore, cognitive scores tended to remain the same or increase during phase 1, and decline over phase 2. This pattern could suggest that either cognitive decline does not begin until later stages, or, because many subjects actually had increases in cognitive test scores over early trials, improvement with learning of the task contributed significantly to the outcome at this stage. Therefore, to minimize the confounding due to contrasting factors (eg, improvement as the result of learning the task versus disease-related cognitive decline), phases 1 and 2 were analyzed separately. When examining longitudinal changes in UPDRS scores and CSF α-syn during phase 1, both subjects who reached the end point and those who did not were included. However, to produce a more homogeneous cohort, only those subjects who reached end point by the end of the open-label trial were included in cross-sectional correlations or longitudinal analyses beginning in phase 2 (ie, all subjects included had started drug therapy at the beginning of this time period).

CSF levels of α-syn were log(10) transformed to compensate for nonnormal distribution of the raw measurements. Longitudinal changes in α-syn were assessed by paired *t*-test. Cross-sectional correlations were determined using partial correlation, controlling for age, sex, and, for cognitive scores, level of education. Individual rates of cognitive decline were calculated by linear regression for subjects with five or more longitudinal test scores. To determine whether CSF biomarker levels could predict longitudinal PD progression, as measured by UPDRS or cognitive scores, linear mixed models were implemented controlling for age, sex, education, PD severity, baseline cognitive score, length of exposure to deprenyl, administration of tocopherol, and follow-up time, with the outcome of interest being the α-syn × follow-up time interaction term. To control for potential effects of levodopa therapy, levodopa equivalent daily dose (LEDD) was determined, as previously described,[20–23] using the following calculation:

$$\begin{aligned}\text{LEDD} = &(\text{regular levodopa dose} \times 1)\\ &+ (\text{slow} - \text{release levodopa}) \times 0.75 + (\text{bromocriptine} \times 10)\\ &+ (\text{pergolide} \times 100) + \text{amantadine} \times 1.\end{aligned}$$

Antihistamine and anticholinergic drugs were not included in the calculation. Mean LEDD reflects the mean (daily) LEDD over the whole phase 2 follow-up period. To control for effects of deprenyl, including variable time of treatment, we also controlled for length of exposure to deprenyl. Statistical tests are two tailed, with significance level set at $P = 0.05$; however, some $P$ values should be interpreted with caution because of the involvement of multiple comparisons in this study. All statistical analyses were performed using IBM SPSS version 19 (Armonk, NY).

## Results

### Demographic and Clinical Measurements

Clinical and demographic data, as well as CSF α-syn levels, at the beginning of phases 1 and 2, are presented in Table 1. CSF samples from 350 subjects met the quality control requirement of Hgb cutoff (≤200 ng/mL) at the beginning of phase 1, as did 354 at the beginning of phase 2. Of these subjects, 266 reached endpoint by the end of the open label trial, and were included in Phase II analyses. Among subjects whose CSF samples met the requirement at the beginning of both phases 1 and 2 (a total of 304 subjects, eliminating subjects in whom at least one CSF sample did not meet quality control), the longitudinal increase (from the beginning of phase 1 to the beginning of phase 2) in total UPDRS score was 15.27 ± 11.98, whereas motor scores increased by 10.37 ± 8.89. Baseline MMSE scores were typically normal, with >84% of the cohort having scores of 28 or higher. CSF levels of α-syn significantly decreased over phase 1 (Figure 1), with a mean longitudinal change of −0.05 ± 0.21 (log-transformed values, paired *t*-test, $P < 0.001$). The longitudinal decrease in CSF α-syn was similar when the sample was restricted to only subjects who reached end point during phase 1 (−0.05 ± 0.22, $P < 0.001$). Cognitive scores typically remained stable or increased slightly over the phase 1 period, and then tended

**Table 1** Demographic Characteristics of Cohort at Beginning of Phases 1 and 2

| Characteristics | Phase 1 | Phase 2 |
|---|---|---|
| Age (years) | 60.90 ± 9.21 | 62.64 ± 9.03 |
| Range | 34–79 | 37–80 |
| Female/male ratio (% male) | 128:222 (63) | 90:176 (65) |
| Duration of disease (years) | | |
| Means ± SD | 2.08 ± 1.39 | 3.80 ± 1.45 |
| Range | 0–7 | 1–8 |
| MMSE score* | | |
| Means ± SD | 28.86 ± 1.44 | 28.74 ± 2.30 |
| Range | 23–30 | 8–30 |
| H&Y | | |
| Median | 1.5 | 2.0 |
| Range | 1.0–2.5 | 1.0–4.0 |
| UPDRS total | | |
| Means ± SD | 23.65 ± 11.70 | 44.97 ± 13.74 |
| Range | 0–63 | 8.5–88.0 |
| UPDRS motor | | |
| Means ± SD | 15.85 ± 8.80 | 30.24 ± 10.41 |
| Range | 0–50 | 4.5–62 |
| α-Syn (ng/mL) | | |
| Means ± SD | 0.63 ± 0.73 | 0.47 ± 0.18 |
| Range | 0.13–8.41 | 0.17–1.09 |
| SDMT | | |
| Means ± SD | 40.21 ± 10.90 | 39.38 ± 11.72 |
| Range | 3–70 | 9–80 |
| SRT-Total Recall | | |
| Means ± SD | 44.88 ± 9.74 | 46.83 ± 10.66 |
| Range | 19–71 | 12–71 |
| SRT-Delayed Recall | | |
| Means ± SD | 7.31 ± 2.67 | 6.87 ± 2.90 |
| Range | 0–12 | 0–12 |
| New Dot Test | | |
| Means ± SD | 12.80 ± 1.46 | 12.65 ± 1.44 |
| Range | 7–14 | 7–14 |

Data are given as means ± SD unless otherwise indicated. Samples with >200 ng/mL Hgb are excluded.
*Available only at phase 1.
H&Y, Hoehn and Yahr.

to decline during phase 2. No differences in α-syn levels were observed between treatment groups at phase 1 or phase 2 (analysis of variance: phase 1, $P = 0.660$; phase 2, $P = 0.939$).

## Association of CSF α-Syn with Motor Symptoms

Cross-sectional correlation of CSF α-syn with UPDRS total and motor scores, controlling for age and sex (both time points), as well as exposure to deprenyl or tocopherol (at phase 2 only), was performed for both the beginnings of phases 1 and 2. No association was found between α-syn and total or motor scores at either phase 1 (UPDRS total correlation, −0.039, $P = 0.471$; UPDRS motor correlation, −0.063, $P = 0.241$) or phase 2 (total, −0.049, $P = 0.359$; motor, −0.051, $P = 0.347$). In addition, the change in α-syn level did not correlate with UPDRS obtained at the beginning of phase 2 (data not shown), controlling for age, sex, and exposure to study drugs.

The predictive value of CSF α-syn was also assessed, using linear mixed models controlling for age, sex, and UPDRS at baseline (for phase 1), or in the 6 months preceding CSF sample collection (for phase 2). For phase 2, exposure to deprenyl or tocopherol in phase 1 was also controlled. CSF levels at the beginning of each phase did not significantly predict UPDRS total or motor progression during phase 1 (total: interaction coefficient, 0.31 ± 0.17, $P = 0.070$; motor: interaction coefficient, 0.22 ± 0.12, $P = 0.055$) or phase 2 (total: interaction coefficient, 0.17 ± 0.11, $P = 0.147$; motor: interaction coefficient, 0.10 ± 0.08, $P = 0.181$). Controlling for exposure to deprenyl in phase 1 and mean (daily) LEDD in phase 2 did not affect the outcome (Supplemental Table S1). The change in α-syn between phase 1 and phase 2 did not predict UPDRS total or motor progression in phase 2, controlling for age, sex, UPDRS, and exposure to study drugs, with or without controlling for mean LEDD (data not shown).

## Association of CSF α-Syn with Cognitive Decline

The cross-sectional association of CSF α-syn with cognitive performance was also examined, considering age, sex, and education, as well as study drug exposure (in phase 2), as covariates (Table 2). At each time point, CSF α-syn was associated only with a single memory test (SRT-Delayed in phase 1; SRT-Total in phase 2).

The relationships between individual α-syn measures and individual rates of cognitive decline (calculated initially using linear regression) for each subject are shown in Figure 2. None of the results demonstrated significant correlations; however, these data do not represent effects observed in the data in aggregate when controlling for potential confounding variables. To account for these variables and assess the value of CSF α-syn measured at the beginning of phase 1 or phase 2 in predicting longitudinal cognitive decline in the following time period, we used linear mixed models, controlling for age, sex, education, exposure to study (ie, deprenyl or tocopherol) or

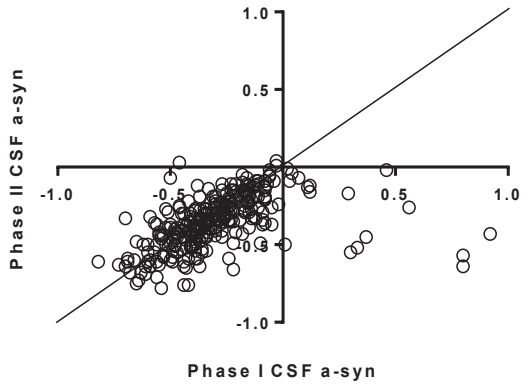


**Figure 1** Log-transformed levels of CSF α-syn at phase 2 as a function of phase 1 level. Line represents no change from beginning of phase 1 to beginning of phase 2.

**Table 2** Cross-Sectional Association of Cognitive Scores with CSF α-Syn

| | Phase 1 | | Phase 2 | |
|---|---|---|---|---|
| Test | Corrected value | *P* value | Corrected value | *P* value |
| SDMT | −0.020 | 0.716 | −0.022 | 0.680 |
| SRT-Total Recall | 0.079 | 0.144 | −0.110 | **0.042** |
| SRT-Delayed Recall | 0.122 | **0.024** | −0.062 | 0.253 |
| New Dot Test | −0.004 | 0.938 | 0.002 | 0.968 |

Correcting for age, sex, and education at phase 1, as well as length of exposure to deprenyl and exposure to tocopherol at phase 2.
Bold indicates significance at the $P < 0.05$ level.

therapeutic (ie, mean LEDD) drugs, and test performance at the beginning of the relevant time period (Table 3). CSF α-syn at baseline did not predict cognitive outcome in any test over phase 1, but CSF values at the beginning of phase 2 predicted outcome over phase 2 in SRT-Total, SRT-Delayed, and New Dot Test. The negative coefficient indicates that a higher marker value tends to predict a greater negative slope (faster decline in cognitive score). Figure 3 shows the modeled cognitive decline over phase 2 for subjects with mean, low, or high (mean, mean ± 1 SD) CSF α-syn at the beginning of phase 2. Thus, the model predicts that subjects with high CSF α-syn show faster deterioration in cognitive performance (Figure 3). To determine whether overfitting contributes to the significance of this result, sensitivity analysis was done by excluding individual variables included in the model. Remarkably, exclusion of age, sex, education, UPDRS, length of deprenyl exposure, tocopherol treatment, and test performance just before phase 2 did not meaningfully alter the test outcome. Notably, the change in α-syn levels from phase 1 to phase 2 did not predict cognitive outcome in any test (data not shown).

To determine whether *ApoE* genotype further affects the relationship, *ApoE* genotype was included in the model, and the three-way interaction between *ApoE* genotype, follow-up time, and CSF α-syn was considered (Table 4). A marginally significant interaction was observed in only one of the eight (four tests in two time intervals) conditions, and inclusion of *ApoE* genotype in the model did not change the conclusion (significant relationship between time and α-syn or not) in any case.

Although it is unclear how levodopa might affect cognitive performance in PD, when drug treatment effects were included in the model, neither mean LEDD main nor interaction (mean LEDD × follow-up time in phase 2) effects were significant, and the model was virtually unchanged when mean LEDD was included (Supplemental Table S1).

## Discussion

The following are the most important findings of this study: α-syn decreased significantly over approximately 2 years of follow-up in patients with PD, but did not predict the worsening of motor symptoms (UPDRS) over phase 1 or phase 2, and CSF α-syn level significantly predicted progression of cognitive decline over the phase 2 follow-up period, but not over phase 1.

α-Syn has consistently been shown to be decreased in subjects with PD compared with controls,[6,7,9,10,24,25] but does not correlate with PD severity in cross-sectional studies, when relatively large cohorts are analyzed.[6,8,11] In this longitudinal cohort, we found a significant decrease over 2 years of PD progression. Considering that these subjects are at early stages of the disease, these results suggest the decreases in α-syn begin fairly early and are likely important in the disease process. Whether this observation can be extended to premotor phases of the disease, making progressive decreases in CSF α-syn, a useful biomarker for premotor PD in at-risk populations,[26,27] should be further probed. Although the cause of lowered CSF α-syn in PD and other synucleinopathies[6,7,9,10,24,25] is not clear, one hypothesis is that α-syn decreases because of its sequestration in Lewy bodies. However, the apparent early onset of changes in CSF α-syn may conflict with this notion, because, if it were true, the change would be expected to follow the progression of Lewy pathological characteristics, which, in fact, reflects the severity of clinical outcome throughout the course of the disease (described later).

A negative, but important, observation is that, although α-syn decreased as the disease progressed, its values did not correlate with UPDRS scores, cross-sectionally or longitudinally. It is notable that even in longitudinal assessments, in which biological deterioration is readily apparent by patients' increasingly severe symptoms, overall decreasing

**Table 3** Predictive Value of CSF α-Syn in Cognitive Decline

| | Baseline: phase 1 | | | Final: phase 2 | | |
|---|---|---|---|---|---|---|
| Test | Coefficient | SE | *P* value | Coefficient | SE | *P* value |
| SDMT | −0.0445 | 0.0737 | 0.546 | −0.1071 | 0.0707 | 0.131 |
| SRT-Total Recall* | −0.1301 | 0.0950 | 0.183 | −0.1240 | 0.0590 | **0.037** |
| SRT-Delayed Recall | −0.0151 | 0.0276 | 0.587 | −0.0516 | 0.0166 | **0.002** |
| New Dot Test | −0.0062 | 0.0154 | 0.686 | −0.0326 | 0.0103 | **0.002** |

See Table 2 for the phase 2 results, with or without controlling for mean LEDD.
*Controlling for age, sex, education, baseline UPDRS, baseline test score, length of exposure to deprenyl, and exposure to tocopherol.
Bold indicates significance at the $P < 0.05$ level.

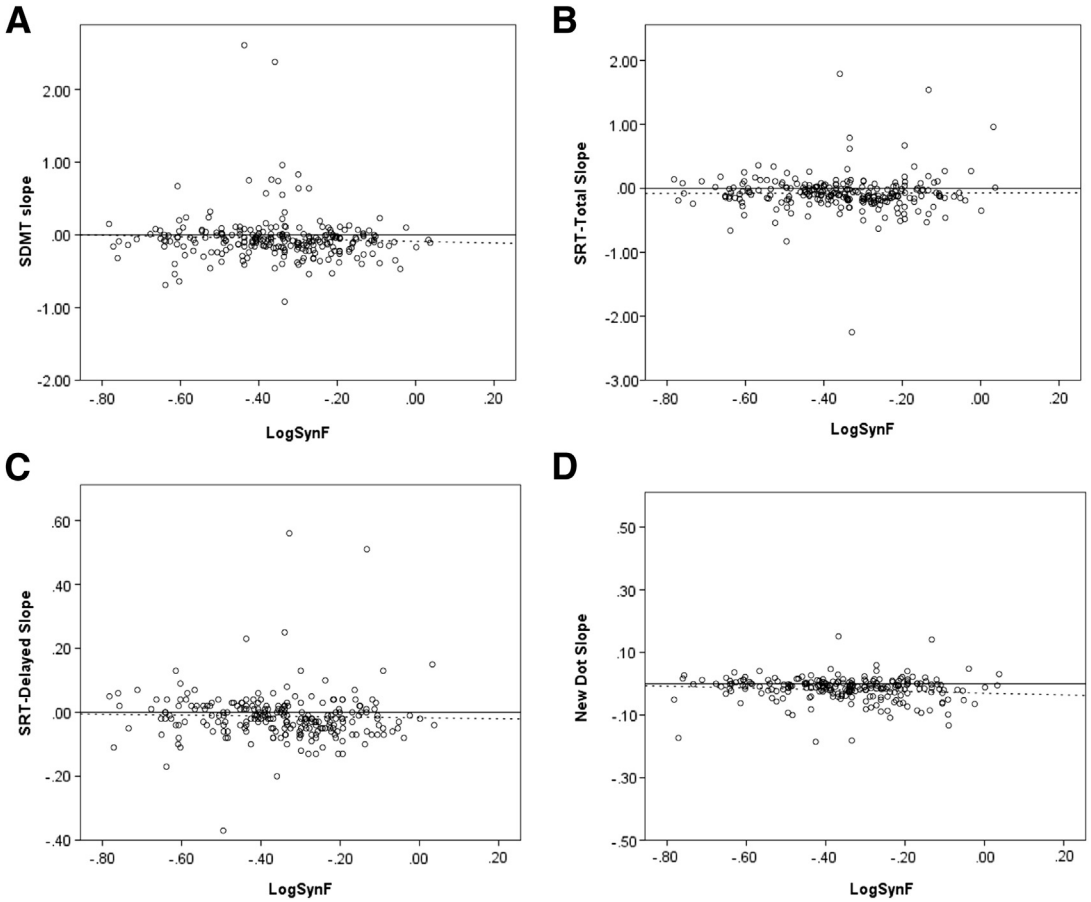


**Figure 2** Individual slope of cognitive decline assessed by SDMT (**A**), SRT-Total (**B**), SRT-Delayed (**C**), or New Dot Test (**D**) as a function of log α-syn at the beginning of phase 2. Includes subjects meeting criteria for phase 2 analysis who had five or more test results for calculation of slope. Dotted lines show correlation. Solid lines represent a slope of 0 (no change in test score). Slopes were generated from linear regression for visualization only, and do not represent effects observed in the data in aggregate when controlling for several confounding variables.

α-syn values did not associate with increasingly severe motor scores, suggesting that there is not a straightforward relationship between CSF α-syn and motor symptoms. As discussed earlier, similar observations have been made in previous cross-sectional investigations,[6,8,11] and have been attributed, at least in part, to drug treatments, because these medicines invariably mask motor scores to some extent. In this study, even at the beginning of phases 1 and 2, when no dopamine drugs were given to any subject, no correlation was observed. One possible explanation is that UPDRS reflects primarily deficits arising from nigrostriatal degeneration, whereas CSF α-syn levels are influenced by the whole brain (and, in fact, the range of cognitive deficits interrogated herein may also reflect widespread cortical involvement). Therefore, α-syn levels may serve better as a proxy for total brain pathological characteristics (see sections dealing with cognitive impairment later) than for motor-specific processes. The caveat, of course, is that drug effects, especially during phase 2, when all subjects received dopamine replacement therapy, mask symptom severity. Although, in theory, this issue can be further addressed by comparing medicated with unmedicated subjects at more advanced stages, its clinical relevance becomes questionable, because all patients with PD eventually require levodopa therapy, as was observed in the DATATOP investigation.

The most important result of this study is the finding that CSF α-syn level predicts cognitive decline. The tests used in this study encompass multiple modes of cognition, including verbal learning and memory, and visuospatial working memory, suggesting that α-syn potentially reflects changes in multiple regions associated with cognition. However, although the trend was in the same direction in phase 1 as in phase 2, it was only significant in phase 2. This could arise from several aspects of the cohorts, particularly the earlier disease stage at phase 1, or the much longer follow-up of phase 2. Alternatively, one might argue that the accelerated cognitive decline during phase 2 might be related to levodopa therapy. Indeed, negative effects of levodopa on cognition have been suggested in animal studies,[28] but the relevance in humans is not clear.[29,30] In the DATATOP cohort, no interaction was detected between LEDD and cognition, and inclusion of LEDD as a relevant variable did not change the conclusions (Supplemental Table S1). Thus, we believe the more significant correlation

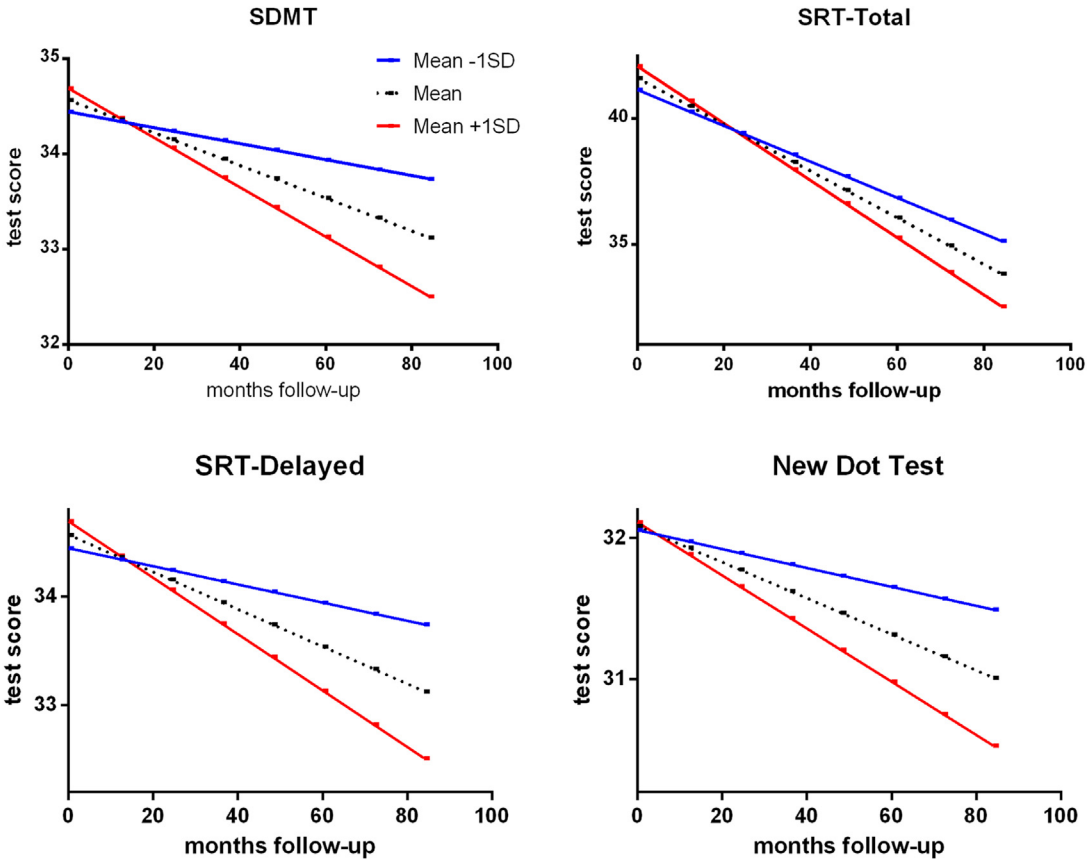


**Figure 3** Modeled cognitive decline over follow-up period for low (means − 1 SD; blue line), mean (black dashed line), and high (means + 1 SD; red line) CSF α-syn.

between α-syn and cognitive decline during phase 2 is likely because of a longer follow-up and disease progression, rather than dopamine replacement therapy.

Whether α-syn plays a direct role in development of cognitive impairment in PD is not known, but given the proposed role of α-syn in synaptic transmission,[31] it is possible that altered α-syn metabolism may be an important factor. Several studies have shown that a greater burden of Lewy bodies in the cortex is associated with dementia.[32–34] Alterations in CSF α-syn have been observed in Lewy body dementia compared with control subjects.[7,8,10] Nonetheless, the direction of the prediction of cognitive decline (ie, the finding that low CSF α-syn predicts slower progression) is somewhat counterintuitive. Several large cross-sectional studies have found that patients with PD and other synucleinopathies have lower levels of CSF α-syn than controls. Therefore, it might be expected that subjects with more severe PD, regardless of whether severity is measured by motor or cognitive scores, might be expected to have the lowest CSF levels if CSF α-syn levels continue to decrease as the disease advances. Although few studies have examined the relationship between CSF α-syn and cognition, one study found a positive correlation between α-syn and MMSE in patients with Lewy body dementia.[35] In contrast, our recent study of α-syn, comparing CSF levels in patients with Alzheimer disease and mild cognitive impairment with controls, found a negative correlation (ie, lower α-syn levels were associated with greater cognitive function).[36] The decreasing level of CSF α-syn in PD may be the result of cellular mechanisms for sequestering α-syn, particularly the pathological soluble species that have been suggested to be the most toxic.[37] However, as previously mentioned, this idea conflicts with the finding that α-syn does not typically correlate well with disease severity, whereas Lewy body pathological features increase with progression. Instead, these results support the hypothesis that the decrease in CSF α-syn is the result of a compensatory process. In this model, those with the lowest levels would be those best able to retain

**Table 4** Three-Way Interaction Effect of *ApoE* Genotype on Model of Cognitive Decline

| | Phase 1 | | | Phase 2 | | |
|---|---|---|---|---|---|---|
| Test | Coefficient | SE | *P* value | Coefficient | SE | *P* value |
| SDMT | 0.081 | 0.270 | 0.763 | 0.096 | 0.197 | 0.628 |
| SRT-Total* | −0.362 | 0.268 | 0.183 | −0.216 | 0.167 | 0.198 |
| SRT-Delayed | −0.134 | 0.084 | 0.113 | −0.098 | 0.048 | 0.042 |
| New Dot Test | −0.038 | 0.046 | 0.405 | −0.019 | 0.030 | 0.533 |

*Controlling for age, sex, education, baseline UPDRS, baseline test score, length of exposure to deprenyl, and exposure to tocopherol.

α-syn, particularly in an environment where some portion of the total α-syn is rendered nonfunctional by aggregation or post-translational modification. Also, they would receive the most benefit from whatever protective physiological effect drives the process, resulting in a CSF analyte level most dissimilar to controls, but enjoying less severe clinical symptoms or progression than those with more normal-appearing CSF. Thus, the retained α-syn would maintain the normal physiological roles of the protein, which, although not fully understood, are suggested to be important for cognitive functions, including memory,[38] through a variety of potential mechanisms, including their proposed role in dopamine metabolism.[31,39–41] Through such a mechanism, damaged or degenerating neurons might maintain their function for a longer period than would those in subjects with less efficient α-syn retention, and subsequent higher CSF levels. Although we cannot determine from the current work which species of α-syn are involved, it does suggest a novel interpretation of lowered α-syn in the CSF of patients with PD, specifically that the driving mechanism may not be minimization of toxicity by pathological species, but retention of functional α-syn, possibly at synapses throughout the brain, even before significant neurodegeneration (Figure 4). Presumably, such a process would be secondary to other disease effects (eg, genetic and environmental factors affecting the production, release, and clearance of α-syn in the brain), but what these processes may be remains to be determined.

In addition to LEDD treatment in all patients with PD, several additional caveats must be considered. First, because the original study was targeted at comparing treatments in patients with PD, no neurologically normal controls were included in the DATATOP study. Ideally, all of the changes we describe herein must be confirmed in cohorts including healthy controls for comparison. However, these results are an important first step. Because of the expense, difficulty, and need for repeated invasive CSF collections in sick subjects required for longitudinal studies of neurodegenerative disease, these results, using available resources, are an encouraging prelude to larger studies. Second, tests to extensively probe multiple modes of cognitive function were not included in the study, and of those that were included, only a few were administered after phase 1. We have limited analysis to those that were continued in phase 2 and, thus, many aspects of cognition were not analyzed in this study. In addition, several measurements were made on the basis of multiple tests, and when multiple comparisons are corrected for, some of the results would not reach statistical significance. That being said, because longitudinal studies of cognition in PD are difficult, and few large cohorts are available, such hypothesis-generating work is vital, and necessary for the development of more targeted studies of future cohorts. Hopefully, the results obtained in such a large cohort can be validated further in a totally independent cohort (eg, those being enrolled in additional large studies, such as the PD Progression Markers' Initiative investigations). These results should be considered for replication when the longitudinal samples become available in the coming years. Finally, although the inclusion criteria required exclusion of subjects with dementia at recruitment, the study began before assessment of mild cognitive impairment (MCI) became standard for studies of early PD. Therefore, some subjects included may have met current definitions of MCI, but that information is not available for inclusion in the analysis. However, several lines of evidence indicate that MCI was not a major contributor to the results

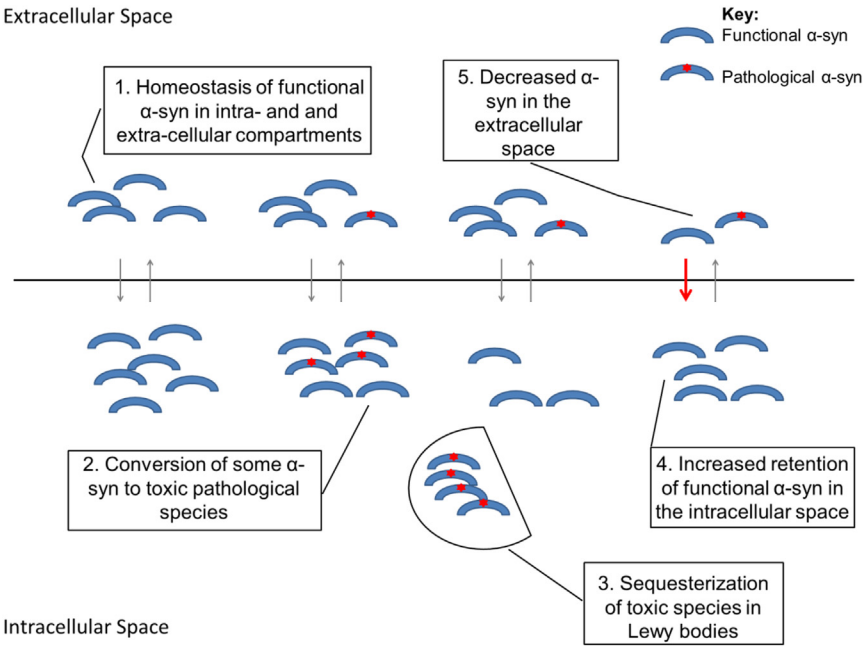


**Figure 4** Hypothesized driving mechanisms for α-syn depletion from CSF in PD. Under normal conditions, brain mechanisms for α-syn production, release, and clearance combine to generate normal α-syn homeostatic conditions (1). In PD pathogenesis, an unknown fraction of α-syn becomes pathological (2). Cells respond to the presence of pathological α-syn by sequestering toxic species in Lewy bodies (3). Less functional α-syn is, therefore, available in the cell. The homeostatic balance is shifted toward retaining more functional protein within the cell, to maintain cellular function (4). The shift in homeostasis results in lower CSF levels of α-syn, but maintains higher (more normal) levels of functional α-syn at the synapse, resulting in improved maintenance of normal synaptic activity (5).

observed in this study. A total of 84% of the subjects had MMSE scores >28 at baseline, showing no obvious cognitive dysfunction, and inclusion of baseline MMSE as a covariable in the predictive models for cognitive decline had little effect on the model (data not shown). Moreover, typical AD markers tau, phosphorylated tau, and $A\beta_{1\text{-}42}$, which are altered in the CSF by the time MCI appears in AD, were examined in the same cohort.[15] In this cohort, AD biomarker levels at baseline and at the beginning of phase 2 were consistent with those in control populations,[15] suggesting that no prominent AD-type pathological feature is present.

In summary, several major observations have been made in this precious longitudinally collected set of CSF samples, along with clinical data, of patients with PD. The most important discovery centers on the finding that CSF α-syn predicts cognitive decline, and may be a useful tool in identifying patients at risk of faster progression of cognitive dysfunction. α-Syn level decreased as PD progressed; yet, it is those with higher α-syn levels who experienced faster cognitive decline. This observation, if confirmed, especially in drug-naïve patients or relevant animal models, could shed more light in understanding PD pathogenesis.

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## Supplemental Data

Supplemental material for this article can be found at *http://dx.doi.org/10.1016/j.ajpath.2013.12.007*.

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