Special Report

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Biomarkers in Medicine

The Systemic Synuclein Sampling Study: toward a biomarker for Parkinson's disease

The search for a biomarker for Parkinson's disease (PD) has led to a surge in literature describing peripheral α-synuclein (aSyn) in both biofluids and biopsy/autopsy tissues. Despite encouraging results, attempts to capitalize on this promise have fallen woefully short. The Systemic Synuclein Sampling Study (S4) is uniquely designed to identify a reproducible diagnostic and progression biomarker for PD. S4 will evaluate aSyn in multiple tissues and biofluids within the same subject and across the disease spectrum to identify the optimal biomarker source and provide vital information on the evolution of peripheral aSyn throughout the disease. Additionally, S4 will correlate the systemic aSyn profile with an objective measure of nigrostriatal dopaminergic function furthering our understanding of the pathophysiological progression of PD.

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The search for an accurate, reliable, early-disease biomarker for Parkinson's disease (PD) from an easily accessible source is a high priority. Aggregated α-synuclein (aSyn) in characteristic Lewy bodies as well as widespread neuritic and presynaptic aSyn deposits [1], in concert with nigral degeneration, is considered the pathological hallmark of PD [2]. It is not currently possible to reliably identify the *in vivo* presence of pathological aSyn, thus a definitive diagnosis is not possible without postmortem examination of the brain [3]. aSyn is found in extracellular biofluids [4] as well as throughout the peripheral nervous system (PNS), especially the autonomic nervous system (ANS) and enteric nervous system (ENS) [5]. Indeed, it has been suggested that peripheral aSyn pathology may precede central pathology (contributing to prodromal symptoms, such as, constipation) and spread to the brain via the dorsal motor root of the vagus nerve [6]. Unlike dopaminergic imaging biomarkers, which are surrogate markers of dopaminergic neuron degeneration, aSyn represents the primary pathology rather than a consequence on neurodegeneration and is therefore less likely to be affected by compensatory responses. Thus, aSyn is more likely to directly reflect overall disease stage, severity and progression. Combined, these factors make aSyn the singularly most promising potential biomarker for PD. However, despite encouraging studies, attempts to capitalize on this promise and develop a biomarker for PD have to date fallen woefully short.

In colon biopsies, acquired by colonoscopy, initial reports suggested 100% sensitivity and specificity to differentiate PD from healthy controls (HC); unfortunately, these results have not been borne out in subsequent studies [7–9] prompting well-received calls for multicenter studies to establish consensus criteria for the standardization of acquisition, visualization and quantification of ENS aSyn [8]. Inconsistency in reported sensitivity and specificity of colonic aSyn likely results from the wide range of sampling regions, depth of biopsy, differing immunohistochemical methods, inadequate blinding

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and differing capabilities of those reviewing and interpreting the results. To this end, the Michael J Fox Foundation for Parkinson's Research recently supported two multicenter studies aimed at determining tissue sample characteristics and immunohistochemical methods to reliably visualize colonic aSyn. These studies suggest that submucosal tissue from the sigmoid colon, when present in adequate volume and when assessed using specific immunohistochemical techniques by accurate observers, may have 100% sensitivity and specificity for pathologically advanced PD subjects as compared with controls [10,11].

Other biopsy sites have not yet undergone multicenter evaluation precluding firm conclusions regarding their usefulness. Both biopsy and autopsy studies regarding cutaneous aSyn are fraught with methodological variability in both acquisition and the analytical methods (including tissue fixation, sectioning methods and antibodies used), yielding wildly inconsistent sensitivity to detect PD ranging from 0 to 100% [5,12–22]. On the other hand, the submandibular salivary gland (SMG) has 74–90% sensitivity in fine-needle biopsy or postmortem tissues and specificity of 80–100% to differentiate PD from HC [23–26]. These findings have been independently approximated by a second group [27], but questions remain regarding the feasibility to obtain biopsies of sufficient quality across multiple trial sites, as needle core biopsies have failed to provide adequate tissue in 25–50% of subjects.

In cerebrospinal fluid (CSF), a decrease in total aSyn and increase in phosphorylated and oligomeric aSyn has emerged as a consistent pattern in PD, with sensitivity and specificity ranging from 61 to 94 and 25 to 64%, respectively [28]. The decrease in CSF total aSyn was recently confirmed in a large multicenter study, but with tremendous overlap of individual values, which would significantly hamper clinical utility [29]. Conflicting results have been reported for plasma aSyn in PD with two studies reporting elevated levels [30,31], one decreased [32] and one no difference [33]. Similarly, one study has noted a significant decrease in salivary aSyn in PD [34], another no significant difference [35] and another noted an increase in the levels of oligomeric aSyn in PD [36]. In all cases, inconsistency in the methodology, including the use of different assays, antibodies and sample preparation protocols complicates comparison between studies. Variability in the performance of the currently available assays is also a major limitation and thus further optimization and characterization of bioassays for detection of aSyn in biofluids will be vital to confirming the early promise of suggested changes in aSyn in PD [4].

aSyn is widely expressed in healthy individuals. Therefore defining what constitutes pathological and physiological aSyn is key to aSyn's success as a biomarker. Lewy bodies have been described in 10–12% of neurologically asymptomatic individuals over the age of 60 [37–39] and studies have observed aSyn phosphorylated at serine 129 (the predominant isoform in Lewy bodies) in tissue from individuals without PD, raising the possibility that phosphorylation of aSyn may be a normal event in aging [40–42]. Some have applied methods to study oligomeric or aggregated forms of the protein, with mixed success. Oligomeric aSyn in CSF, saliva and red blood cells has been shown to be increased in PD [33,36,43,44]. However there are conflicting reports of increased or no change in plasma oligomeric aSyn in PD [28]. Efforts to detect aggregated aSyn in tissues have failed to differentiate individuals with PD from HC [42]. Evidence that pathological aSyn may possess prion-like qualities suggests that repositioning techniques from the prion field may yield better selectivity for pathological forms of aSyn in PD, as evidenced by a recent report employing a seeding-based assay to successfully differentiate aSyn in CSF from individuals with PD compared with HC [45,46]. The search for further disease-specific post-translational modifications of aSyn continues and indeed may result in changes in the definition of pathological aSyn.

A final hurdle in realizing the potential of aSyn as a PD biomarker is a lack of understanding of the distribution of peripheral aSyn within a given individual and the evolution of aSyn burden over the course of disease. The SMG reportedly possesses the highest phosphorylated aSyn burden in Lewy body diseases compared with multiple other biopsy-accessible sites [5]. However, reports of a centripetal spread of aSyn pathology raise the possibility that aSyn pathology may only be evident in proximal regions early in the disease and disappears as axons die back during disease progression [47,48]. Autopsy and biopsy studies including early- and late-stage PD subjects suggest that this does not occur [23,25,26] but serial biopsies of the same subject are required to assess whether PNS aSyn pathology progresses or regresses with disease duration. For skin biopsies, the extensive availability of possible anatomical sites complicates the analysis. Some studies have suggested skin biopsies from more proximal regions or from anhidrotic skin areas [20,49], may have more prevalent aSyn deposition in PD [12,16,17], highlighting the need to assess the burden of aSyn both topographically and temporally within a given individual.

The Systemic Synuclein Sampling Study (S4) aims to address many of these issues and, through standardization and monitoring of collection, processing and analysis of samples, provides much needed clarity to the field regarding the feasibility of detecting peripheral aSyn as a robust and reliable biomarker for PD.

Study assessments

S4 is a multicenter study that samples biofluids (CSF, saliva and blood) and peripheral tissues (skin, colonic submucosa and SMG) in individuals with PD (across the disease spectrum) and HC subjects (Table 1). All study procedures will be carried out in compliance with guidelines on human experimentation as well as protocol approval by a local Institutional Review Board.

Study cohort

The study cohort is outlined in Table 2. Up to 100 subjects will be enrolled across six clinical research centers (Supplementary Material 1), with a goal of having 60 individuals with PD and 20 HC subjects complete at least two biofluid and two tissue samplings. Individuals with any condition or therapy that would preclude the safe performance of study procedures will be excluded (Supplementary Material 2). Screening includes procedures to evaluate eligibility and clinical characterization of PD (Table 1).

Biofluid sampling

Detailed protocols for biofluid collection (whole blood, plasma, serum, CSF and saliva) can be found in the S4 biologics manual [50]. All biofluids are collected from 8 to 10 am following an overnight fast, or adherence to a low-fat diet. aSyn contamination from hemolysis and incomplete clearance of erythrocytes and platelets is a major confounding factor in biofluid studies to date [51]. S4 researchers are instructed not to agitate the buffy coat and packed blood cells after centrifugation and to avoid disturbing the pellet when aliquoting. Hemoglobin measurements and blood cell counts of all collected fluids are part of the analysis plan.

Skin biopsy

Three-millimeter punch biopsies of the skin are obtained under local anesthesia (lidocaine). To control for the heterogeneous distribution of cutaneous aSyn, the location of the biopsies is standardized. Two biopsies from the paravertebral region (adjacent to the cervical spine) will be taken at the C8 level 3 cm from the midline. Thigh biopsies will be taken 15 cm above the patella. One study obtained 100% sensitivity by analyzing two paravertebral skin samples [12]; for this reason, and to protect against loss or inadequacy of a single sample, S4 will collect two-punch biopsies per site. In spite of positive findings using Zamboni fixative, large-format thick frozen sections and immunofluorescence [14,16], S4 skin biopsies will be formalin fixed and paraffin embedded in order to enhance reproducibility and feasibility of scaling to large multicenter studies with clinical-grade assays.

Colon biopsy

Biopsies are obtained by flexible sigmoidoscopy performed by a gastroenterologist with optional conscious sedation and optional enema for bowel preparation. The biopsy location (sigmoid colon) represents a compromise between the well-established rostrocaudal gradient in ENS aSyn deposition [9], and the ease and availability of flexible sigmoidoscopy as compared with full colonoscopy with bowel preparation and sedation, which would increase participant burden and be impractical for a large multisite clinical trial. Likewise, the depth of biopsy considers that it is unsafe to biopsy the muscular layer and Auerbach's plexus where pathology is most consistently detected [7]. Sample collection targets inclusion of the submucosal plexus which is safely accessible by routine biopsy. Due to the diffuse distribution of the submucosal neuronal network and the inadequate volume of submucosa obtained by one or two biopsies [10], eight separate biopsies will be collected using radial jaw forceps with needles (Boston Scientific, MA, USA, M00513410), to ensure sufficient intact sample is obtained to gain an accurate representation of the aSyn present.

Submandibular gland biopsy

S4 is collecting 4–5 SMG needle core biopsies under local anesthesia using a 16-gauge core biopsy instrument (Bard Medical, GA, USA, MC1610). The number of biopsies and diameter of the needle are based on past biopsy studies [23,27] and represent a compromise between invasiveness and the need to obtain sufficient tissue. To minimize the limitations, the procedure is carried out by specifically trained otolaryngologists with optional ultrasound guidance.

Standardization of data/biospecimen acquisition

Given the high variability in peripheral aSyn as a biomarker for PD in the current literature (sensitivity and specificity ranging from 0 to 100%), standardization of collection procedures, processing and analysis are pivotal to S4. All procedures are conducted by local specialists (otolaryngology, gastroenterology, nuclear medicine and neurology). Training calls are held with complementary videos detailing each study procedure [52]. All tissues have standardized fixation times. Hematoxylin and eosin sections are prepared and reviewed by the histopathology core (Banner Sun Health Research Institute) to document the volumes of critical tissue components (mucosa and submucosa for colon; glandular and extraglandular tissue for SMG; and epidermis, dermis and hypodermis for skin). Reviews are communicated back to sites to provide direction for succeeding biopsies. Sites are continu-

Table 1. Schedule of events.

Visit description	Screening visit	Biofluid collection and skin biopsy visit	Colon biopsy visit	Submandibular gland biopsy visit
Written informed consent	X			
Screening demographics	X			
Assign unique ID	X			
Review inclusion/exclusion criteria	X	X	X	X
Medical history	X			
Medical history of PD	X			
Review family history for PD	X			
General neurological examination	X			
Physical examination	X			
ECG	X			
Vital signs	X	X[†]	X[†]	X[†]
MDS-UPDRS (part IV will not be done for healthy controls or early PD subjects)	X[‡]			
Hoehn and Yahr (all subjects)	X			
Schwab and England (PD subjects only)	X			
SCOPA-AUT (all subjects)	X			
UPSIT administration (all subjects)	X			
Montreal Cognitive Assessment (all subjects)	X			
PD stage assignment	X			
Clinical safety labs	X[§]			
DatSCAN SPECT imaging	X			
Blood collection for safety evaluation (Chem-20, CBC, PT/PTT)	X			
Blood collection for whole blood, plasma, serum, DNA and RNA		X[¶]		
Saliva collection		X[#]		
LP procedure		X[††]		
Skin biopsy procedure		X[‡‡]		
Colon biopsy procedure			X[§§]	
Submandibular gland biopsy procedure				X[¶¶]
Concomitant medications	X	X	X	X
AE assessment	X[##]	X[##]	X[##]	X[##]

[†]Vital signs (blood pressure, heart rate, respiratory rate and temperature) will be obtained prior to the study procedure and at the completion of the study procedure for each of the following procedures: DAT-SPECT, lumbar puncture, skin biopsy, colon biopsy and submandibular gland biopsy.
[‡]MDS-UPDRS can be done at any visit.
[§]Clinical safety labs will include Chem-20, CBC, PT and PTT.
[¶]Blood collection (~46 ml) will occur preferably in the fasting state. If fasting is not possible, the subject may have a low-fat/low-lipid meal.
[#]Approximately 5 ml of saliva will be collected via passive drool. Subjects should refrain from food intake, drinking liquids and using oral hygiene products for at least 1 h prior to saliva collection.
[††]Lumbar puncture will be performed in the morning, preferably in a fasted state. If fasting is not possible, the subject may have a low-fat/low-lipid meal. Approximately 20 ml of CSF will be collected.
[‡‡]Skin biopsies, using a punch biopsy under local anesthesia, will be obtained in the paravertebral region and distal thigh. Four biopsies will be obtained (two from each location).
[§§]Colon biopsy will be performed through flexible sigmoidoscopy. Eight biopsies will be obtained.
[¶¶]Submandibular gland needle biopsy will be performed using local anesthesia. Four biopsies will be obtained.
[##]AEs will be collected at the visits and during follow-up phone calls after each procedure. A phone call will occur at 7 days (±2 days) following DAT-SPECT imaging and lumbar puncture procedures and at 7 days (±2 days) following skin biopsy, colonic biopsy and submandibular gland biopsy.
AE: Adverse event; CBC: Complete blood count; CSF: Cerebrospinal fluid; DAT: Dopamine transporter; LP: Lumbar puncture; PT: Prothrombin time; PTT: Partial thromboplastin time; SPECT: Single-PET.

Table 2. Systemic Synuclein Sampling Study cohort details.

Feature	Healthy controls	Early untreated PD	Moderate PD	Advanced PD
n	20	20	20	20
Age (years)	≥50	≥40 at diagnosis		
Diagnosis	No significant neurological disorder†	Clinical diagnosis of PD (bradykinesia plus either rest tremor or rigidity)‡		
DAT deficit by SPECT	Absence of DAT-SPECT deficit at screening	Presence of DAT-SPECT deficit at screening		
Years since diagnosis	NA	<2	2–5	>5
Dopamine replacement therapy	NA	None	Yes	Yes
Motor fluctuations or dyskinesias	NA	None	None	Yes

†Exclusion criteria for HC subjects include a family history of PD in any first-degree relative, any significant neurological disorder, a Montreal Cognitive Assessment score <26, a diagnosis of REM sleep behavior disorder, primary dystonia, restless legs syndrome, essential tremor or other movement disorder.
‡PD subjects with any other significant neurological disorder, significant autonomic dysfunction and/or any other features of atypical parkinsonism will be excluded.
DAT: Dopamine transporter; HC: Healthy control; PD: Parkinson's disease; NA: Not applicable; REM: Rapid eye movement; SPECT: Single-PET.

ally monitored for compliance allowing retraining if required during the collection period.

S4 objectives & analysis

The primary objective of S4 is to evaluate aSyn in each peripheral matrix as a potential marker for patient selection/enrichment for future clinical trials. Most critical will be the sensitivity and specificity for PD, but inter-reader variability will also be important. The major objective is to identify a test that will improve the efficiency of clinical trials by substantially increasing the probability that included subjects will have aSyn pathology, and by decreasing the individual outcome variability and hence required subject numbers to detect a clinically significant effect. Additionally, the anatomical distribution of aSyn will be compared between individuals with PD and compared with biofluid measures. As S4 is the first study to evaluate aSyn in multiple tissues and biofluids within the same subject and between different groups, formal sample size estimates were not attempted. However, we estimate, based upon CSF aSyn data from the Parkinson's Progressive Markers Initiative [29], that 20 subjects per group will have 80% power to detect a difference of 40% or more between HC and one of the later PD cohorts versus the early PD cohort ($p < 0.05$).

Biofluid analysis: biofluids will be analyzed using the most optimal, quantitative and semiquantitative assays for total aSyn and other aSyn species (as well as other markers) available at the time of analysis. Extracted DNA and RNA will be used for *SNCA* sequencing and aSyn transcript isoform sequencing and quantification. Currently several assay development and comparison studies are being conducted to prepare for S4 sample analysis. All S4 biofluid collection protocols are based on the methods of two large prospective studies: Parkinson's Progressive Markers Initiative and BioFIND [53], which also are very similar to those used by the PD Biomarker Program [54] facilitating the comparison of S4 samples to these other multicenter PD biomarker cohorts.

Tissue analysis: immunohistochemistry for aSyn will be performed by histopathology experts blinded to diagnosis using the most optimal techniques available at the time of analysis. Several assay development and comparison studies are underway. aSyn burden will be expressed as: simply positive or negative (whether any two slides are positive out of all examined); by total percentage of slides examined that are positive; and by site of the highest density of aSyn positive fibers. Our previous work has demonstrated large inter-rater variability in assessing aSyn in tissues highlighting the need for provision of detailed rating templates and in-person training of raters [10,11]. Tissue-specific templates will be developed for semiquantitative grading of aSyn fiber density. All of these will define specific staining as that with morphology consistent with neuronal tissue elements [10].

Secondary objectives include assessment of the fraction of biopsies with inadequate tissue and a comparison of aSyn load among the tissues and fluids in the PD cohort subdivided into three clinical stages using trend tests (continuous or categorical, as appropriate). A strong aspect of S4 is the ability to compare the aSyn load among the tissues and fluids with disease severity based on striatal degeneration measured by dopamine transporter single-photon emission tomography (DAT-SPECT) across the PD groups in addition to comparing PD and HC groups using an appropriate generalized linear model. Adverse events are recorded for 7 days following each study procedure to provide important information regarding the safety and tolerability for future trial design. All statistical analyses will be completed by the S4 statistical core (University of Iowa).

Discussion

S4 is uniquely designed to expand our understanding of the feasibility and utility of assaying peripheral aSyn as an *in vivo* biomarker for PD. To date, studies have evaluated either a single biofluid or tissue matrix, whereas S4 will evaluate multiple tissues and biofluids within the same subject providing valuable information on the peripheral aSyn profile in PD and potentially revealing the optimal matrix (the measurement with the smallest required sample size to detect a clinically significant effect) to evaluate in future clinical trials. Currently the utility of detecting aSyn as a progression biomarker at different stages of PD has not been extensively studied and there is a great need for additional markers being identified for PD risk, progression and severity. Studying individuals in both early and advanced disease will provide important insight into the evolution of peripheral aSyn burden throughout the spectrum of disease severity. Furthermore, S4 is the first study to correlate the systemic aSyn profile with an objective measure of nigrostriatal dopaminergic function (DAT-SPECT), allowing the evaluation of disease severity without the confounding effect that antiparkinsonian medications have on clinical scales.

Development of an aSyn biomarker through S4 offers the exciting possibility to further our understanding of the pathophysiological progression of PD. A popular hypothesis posits that PD may begin with pathological aSyn deposition in the ENS, which initiates a spreading of pathology in the brain via the dorsal motor root of the vagus nerve, although this remains to be proven [55,56]. Alternately, PD may begin in the olfactory bulb with aSyn spreading centrally and peripherally from there [57,58]. Correlating peripheral aSyn with striatal DAT-SPECT deficit at different stages of disease will provide clarity regarding the hypothesized centripetal spread of PD pathology, which currently is limited to case reports in cardiac autonomic nerves [47,48], and at odds with biopsy and postmortem studies that have thus far failed to find evidence of a disease duration-dependent regression of peripheral aSyn pathology [3,5,6,25,26].

PD is one of several synucleinopathies which share common neuropathological hallmarks and have overlapping clinical and cognitive symptoms which can lead to misdiagnosis especially in early-disease stages [3]. To date, literature regarding peripheral aSyn in Lewy body dementia, multiple system atrophy and PD with dementia is limited. Some studies suggest that peripheral synucleinopathy may be less prevalent in multiple system atrophy than other synucleinopathies [59]. Conversely, we have recently described the presence of pathological deposits of aSyn in the submandibular gland in 71% of subjects with dementia with Lewy bodies (DLB) [25]. S4 focuses on PD, and is therefore not designed to test the potential of peripheral tissue to distinguish between PD and other synucleinopathies. However, this issue is clearly critical to the future application of peripheral aSyn for both diagnostic/prognostic purposes and clinical trials. Future studies will need to ascertain the ability of surveying peripheral aSyn to provide a differential diagnosis among PD and other synucleinopathies.

The optimal assays for detection of peripheral aSyn in S4 are still being determined and are the subject of several ongoing studies. Indeed this is a rapidly developing field with new assays being regularly reported. Importantly, methods will be sought that reduce or eliminate signal from physiological aSyn to negate the need for a threshold to distinguish between physiologic and pathologic levels of the protein that otherwise complicates the biomarker-related utility of aSyn. Analysis of biological fluids will leverage currently available assays (for total aSyn, phosphorylated and oligomeric aSyn). The different available assays will be validated by round-robin experiments with centrally provided samples shared with different laboratories. The comparison of aSyn measured by ELISA and mass spectroscopy is currently being investigated by a Michael J Fox Foundation for Parkinson's Research initiated consortium [60]. Importantly, S4 has paraffin embedded all available tissue, therefore mass spectrometry with the tissue will not be part of S4, but is under consideration to be added onto any future cohort studies. Additionally studies are currently underway to identify many post-translational modifications of aSyn in PD samples (e.g., phosphorylation, ubiquitination, tyrosine nitration, truncation by proteases) and potential of other proteins (e.g., tau) [61]. Once these are identified, and assays are developed, the S4 samples will be tested. Furthermore, it is anticipated that future studies may include the use of techniques directed toward oligomeric forms of aSyn [30,62,63], methods assessing the seeding qualities of peripheral aSyn, such as, the real-time quaking-induced conversion assay [46,64], methods studying different isoforms or species of aSyn [65] and the study of more relevant fluid compartments, for example, exosomal aSyn for quantification in biofluids [28,66]. Indeed it is hoped the tissue and biofluid samples from S4 will serve as a repository for future analyses exploring new methods of detecting aSyn.

Conclusion

The search for a safe, reliable and inexpensive biomarker of PD remains a major unmet need and is currently a high priority in PD research. Through the evaluation

of the distribution of aSyn pathology in multiple tissues and biofluids in individual subjects across a spectrum of PD and HC at a single time point, S4 aims to identify the optimal surrogate marker for PD. The development of a peripheral aSyn biomarker would provide a valuable tool for confirming the diagnosis of PD, and possibly identification of the disease in its earliest stages, and provide a potential means of monitoring efficacy of potential disease modifying agents.

Future perspective

In the next 5–10 years it is likely that several aSyn anti-aggregation/sequestration strategies, currently in preclinical development, will be ready for testing in clinical trials. Indeed, passive immunotherapy is already under clinical trial. It is hoped that S4 will contribute an accurate picture of the peripheral aSyn burden across the clinical spectrum which will be critical to determine the utility of peripheral aSyn as a clinical trial outcome measure. Furthermore, by employing standardized and monitored collection, processing and analysis methodology, it is hoped that S4 will establish well-defined quantitative biomarker outcomes that are consistent and demonstrate reproducibility among multiple research sites and demonstrate feasibility of collecting adequate high-quality samples across multiple sites as would be required for future large-scale clinical trials. Successful identification of aSyn-targeted biomarkers will also open up the tempting possibility of serial sampling in future trials evaluating potential disease-modifying therapies. Finally, S4 could pave the way for future study of the peripheral aSyn profile in prodromal PD by including populations at increased risk to develop PD. Clearly much work is required before such studies can be performed, but certainly S4 represents a promising step in the right direction.

Supplementary data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/bmm-2016-0366

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Executive summary

Background

- The search for a reliable, early-disease biomarker for Parkinson's disease (PD) that reflects underlying pathology but is from an easily accessible source is a high priority in PD research.
- α-Synuclein (aSyn) is the singularly most promising potential biomarker for PD but attempts to capitalize on this promise and develop a biomarker for PD have to date come up woefully short.

Methods

- The S4 is a multicenter cohort study that, through standardization and monitoring of collection, processing and analysis of samples, provides much needed clarity to the field regarding the feasibility of detecting peripheral aSyn as a robust and reliable biomarker for PD.
- S4 samples biofluids (cerebrospinal fluid, saliva and blood) and peripheral tissues (skin, colonic submucosa and submandibular salivary gland) in individuals with PD (across the disease spectrum) and healthy control subjects.

Discussion

- S4 is the first study to evaluate aSyn in multiple tissues and biofluids within the same subject providing valuable information on the peripheral synuclein profile in PD and potentially revealing the optimal matrix to evaluate in future clinical trials.
- Studying individuals in both early and advanced disease will provide an understanding of the evolution of peripheral aSyn burden throughout the disease course.
- S4 is the first study able to correlate the systemic aSyn profile with an objective measure of nigrostriatal dopaminergic function as assessed by dopamine transporter imaging, which provides an exciting opportunity to further our understanding of the pathophysiological progression of PD.
- S4 aims to establish well-defined quantitative biomarker outcomes that are consistent and demonstrate reproducibility among multiple research sites and laboratories for patient selection/enrichment for future clinical trials.

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• of interest; •• of considerable interest

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