A Biological Definition and Integrated Staging System of Neuronal alpha-Synuclein Disease

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Abstract

Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB), and related disorders share the same underlying neurobiology characterized by Lewy pathology reflecting neuronal aggregates of misfolded alpha-synuclein, but they are currently defined by clinical diagnostic criteria. We propose a biological definition for "Neuronal alpha-Synuclein Disease (NSD)" including all clinicopathological entities associated with neuronal-predominant alpha-synuclein (n-asyn) aggregation. NSD is defined by the presence of specific and pathologic n-asyn species by in-vivo tests. This biologic definition is independent of the presence of clinical features, or if present, of the specific clinical syndrome. We further propose that individuals with n-asyn are at high risk for developing dopaminergic neuronal dysfunction (D), a second key biologic anchor for NSD. The Neuronal Synuclein Disease Integrated Staging System (NSD-ISS) integrates these biological anchors (S and D) and degree of functional impairment caused by motor, cognitive or other non-motor signs. Individuals in stage 0 and 1 are free of clinical signs and symptoms and can be diagnosed either by presence of fully penetrant pathogenic variants (G) in *SNCA,* the gene encoding alpha-synuclein (Stage 0), n-asyn alone (Stage 1A) or in combination with dopaminergic neuronal dysfunction (Stage 1B). The presence of clinical manifestations marks the transition to Stage 2 and beyond. Stage 2 is characterized by subtle clinical signs but no functional impairment, with either n-asyn (S) alone (Stage 2A) or in combination with dopaminergic neuronal dysfunction (Stage 2B). Stages 3-6 require the presence of both biomarkers and stage-specific increase in severity of functional impairment. The NSD-ISS provides a comprehensive biologically-based framework essential to advance biologically-targeted therapeutic development. The NSD-ISS is expected to evolve as additional biomarkers emerge. The NSD-ISS will inform our understanding of early disease and accelerate development of disease-targeted therapies, enabling interventional trials before the onset of clinical symptoms.

Introduction

Neuronal aggregates of misfolded pathological species of alpha-synuclein (asyn) in Lewy bodies and Lewy neurites are the pathological hallmark of Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB) and related conditions[1]. Yet, these alpha-synucleinopathies are currently diagnosed based on traditional clinical criteria. These well accepted clinical definitions may include non-specific signs and symptoms and result in heterogenous clinical cohorts. Further, definitions that rely solely on clinical features are inherently flawed because they are unable to identify disease during the early stages of neurodegeneration prior to symptom onset. A biologic definition for alpha-synucleinopathies would inform our understanding of early disease, reduce disease heterogeneity, and provide a framework to track disease progression that would accelerate therapeutic development.

We propose that recent data demonstrating that neuronal alpha-synuclein (n-asyn), previously only measured post-mortem, can be reliably detected *in vivo*[2-5], enables a paradigm shift to a biologic definition for alpha-synucleinopathies. We put forward a new term for all neuronal alpha-synucleinopathies, Neuronal alpha-Synuclein Disease (NSD), defined by the presence of neuronal alpha-synuclein (S) in vivo. We further propose that individuals with n-asyn are at high risk for developing dopaminergic neuronal dysfunction (D), a second key biologic anchor for NSD. Defining NSD by its biology is crucial to further understanding of disease pathophysiology, enabling biology-specific therapeutic development[6] and therapeutic intervention prior to symptom onset to potentially prevent/halt progression[7], and detecting biologically-defined subsets[8].

Two centuries after PD was first described[9] and three decades since the term DLB was proposed[10], the field now has the knowledge and tools to define NSD based on biology and conceptualize a biological staging system for NSD. The NSD Integrated Staging System (NSD-ISS), rooted in biology, builds on similar efforts in other neurodegenerative diseases, including Alzheimer's Disease (AD)[11] and Huntington's disease (HD)[12]. The NSD-ISS identifies disease at the earliest stage of pathology and enables its staging from the period prior to symptom onset to development of clinical functional impairment. The biologic definition and staging of NSD are expected to evolve as additional data emerge, but the proposed NSD staging framework is a crucial step to advance NSD research and therapeutic development.

Process

In 2022, in the setting of a broad consensus among a range of stakeholders that biological definition and staging of alpha-synucleinopathies was critical to advancing therapeutic development[13], a working group was assembled under the auspices of The Michael J. Fox Foundation for Parkinson's Research (MJFF). This working group includes international neuroscience and clinical experts, industry sponsors, disease focused non-profit organizations, regulatory authorities, and representatives of the patient community. Focusing on a data driven approach, the objectives were to: (1) develop a biologic definition for disease; (2) develop a framework for a disease staging platform that delineates disease stages to accelerate targeted therapeutics (3) identify key gaps in knowledge. Following a series of virtual meetings, a face-to-face conference was held in January 2023. Following this conference and a series of virtual meetings, seven international non-profit organizations, including MJFF, supported a face-to-face roundtable and subsequent virtual summit. These events were held in April 2023 with diverse stakeholders, including an expanded group of global NSD neuroscience and clinical experts, patient community, public-private partnership groups, representatives from industry and regulatory agencies, and additional patient advocacy groups. Several key revisions to the NSD-ISS resulted from the open discussion.

Unifying Terminology

Neuronal alpha-synucleinopathies, defined by accumulation of disease-specific[14-16] pathological asyn predominantly in neuronal cell bodies and neurites (Lewy bodies and Lewy neurites), may be asymptomatic or may manifest clinically with parkinsonism, cognitive impairment, and an array of other motor and non-motor manifestations. Based on the sequence and progression of clinical signs/symptoms, individuals have been designated, with varying terms[17-24], such as incidental Lewy body disease (LBD)[25-28], preclinical (without clinical features), premotor, Pure Autonomic Failure (PAF), idiopathic REM-Sleep Behavior Disorder (iRBD), other prodromal (early signs/symptoms, not yet fulfilling clinical diagnostic criteria for a disease), or as having a possible or probable clinical diagnosis of PD, PD dementia (PDD), or DLB.

NSD is proposed *as a new term defined by biology to encompass all neuronal alpha-synucleinopathies.*

NSD does not include Multiple System Atrophy (MSA), an alpha-synucleinopathy characterized by predominantly glial asyn deposition[1]. Current evidence[29-31] suggests that different molecular 'strains' of pathological asyn distinguish alpha-synucleinopathies with neuronal deposition of aggregates versus predominantly glial asyn pathology, as such indicating a different biology in MSA. Although the principles of the NSD-ISS may apply to MSA, the evidence base for a biological definition and in vivo biomarkers characterization are still less advanced in MSA, which is therefore not included here.

Search strategy

PubMed was searched for articles pertaining to: (i) diagnostic criteria for Lewy body disorders (ii) the role of asyn in pathophysiology, (iii) other relevant molecular pathways, including genetics, (iv) *in vivo* biomarkers of asyn, (v) dopaminergic dysfunction and biomarkers for it, and (vi) clinical manifestations of alpha-synucleinopathies across the continuum. Articles were selected based on methodology/level of evidence and relevance. Additional references were nominated from working group members.

NSD Definition

Central tenets of the biologic definition of NSD are: (1) the *disease* is defined biologically based on objective *in vivo* biomarkers in living people (2) the disease can be diagnosed in absence of clinical manifestations and (3) clinical manifestations in the absence of biomarkers do not establish the disease.

NSD is defined by presence of n-asyn (S) and stage-dependent evidence of dopaminergic neuronal dysfunction (D). In addition, presence of fully penetrant pathogenic variants in the *SNCA* gene (G) is sufficient for NSD diagnosis. The current measures used to determine an individual's S or D status are categorical (positive/negative) but are anticipated to become quantitative as the field evolves.

The NSD-ISS applies to the majority (>90%) of individuals diagnosed with PD and DLB based on current clinical diagnostic criteria[2-4].

There are individuals that are n-asyn negative (S-) but have evidence of dopaminergic dysfunction (D+)[2]. These individuals do not have NSD; but rather a distinct biology and are defined separately (see below).

S/D/G anchors of the NSD-ISS: description of *in vivo* biomarkers

S anchor: n-asyn

Presence of n-asyn (S+) is the defining feature of NSD and key to the NSD-ISS staging framework.

Over a century ago, Lewy bodies were identified as the pathologic hallmark of disorders now encompassed in the definition of NSD[32]. Landmark discoveries included identification of the p.A53T *SNCA* variant as a cause for PD and determination that asyn was the core constituent of Lewy bodies and Lewy neurites[33]. The key role of n-asyn pathology in NSD is well established based on extensive pathologic[1,33-36], molecular and genetic evidence[1,37-40] in animal models[1,41,42] and in humans[1-4].

Efforts to identify and measure pathological asyn *in vivo* have been underway for decades. The development, in 2016, of an assay with high sensitivity and specificity for n-asyn[43], now known as seed amplification assay (SAA)[3,44,45] is a major advancement for the field. Cerebrospinal fluid (CSF) SAA has been validated as a robust biomarker of n-asyn[2-4]. This assay is positive in >90% of autopsy confirmed cases of PD and DLB[5,46] and identifies individuals with clinical signs/symptoms of PD and DLB with high accuracy[2-4]. SAA can also detect n-asyn in individuals with iRBD, olfactory or autonomic dysfunction, or genetic risk who are more likely to progress to clinical PD or DLB[2,47-49]. Importantly, this assay distinguishes n-asyn from other asyn forms, such as those associated with the predominantly glial pathology of MSA[30,50].

Standard operating procedures and best practices for biospecimen handling[51] are critical for accurate application of asyn SAA. Currently, the most widely-applied assay[2] defines positivity based on maximal fluorescence emitted during aggregation and requires consistency across technical replicates. At present, CSF SAA is the measure of n-asyn with the highest level of evidence. It is expected that other n-asyn biomarkers will emerge that will be incorporated into future iterations of the staging system, including SAA in other matrices including blood and peripheral tissue[3,41,52,53], measures of disease-specific post-translational modifications of asyn, and exosome-derived biomarkers[41]. A major goal is to develop quantitative measures of n-asyn. Intensive efforts are also underway to develop PET tracers for accurate *in vivo* detection of n-asyn[54].

D anchor: dopaminergic neuron dysfunction/ degeneration

Degeneration of substantia nigra dopaminergic neurons (D) is a core lesion and a second defining feature of NSD.

Loss of midbrain dopaminergic neurons was identified as a key pathology in PD about 75 years ago and soon after dopamine replacement was demonstrated as an effective therapy for PD[55]. There are extensive data showing that dopaminergic dysfunction is present in an overwhelming majority of S+ individuals with motor, cognitive, or other non-motor signs/symptoms of NSD[2,56-60].

Molecular imaging of the dopamine system has been widely used to detect dopamine dysfunction in life. Dopaminergic imaging with fluorodopa or tracers for the dopamine transporter (DAT) or

vesicular monoamine transporter (VMAT) all effectively mimic striatal pathologic change in dopaminergic regions, showing the expected asymmetric, rostral-caudal striatal loss[61,62]. In addition, DAT binding correlates with neuronal density[63] and nigral cell counts[64] in brains of individuals with neurodegenerative disorders. Importantly, over 88% of cases of DLB diagnosed based on clinical diagnostic criteria or on pathology have abnormal DAT single photon emission tomography (SPECT)[56-58].

Studies have clearly demonstrated that DAT loss occurs before functional impairment, and further that individuals without functional impairment with DAT deficit are likely to progress to develop functional impairment within 3-5 years. For example, reduced DAT binding in people with olfactory dysfunction or iRBD predicts motor and cognitive progression[65-69]. Similarly, individuals with motor signs/symptoms without DAT deficit[70] are unlikely to have n-asyn[2] and are unlikely to progress to more severe functional impairment.

Currently, DAT SPECT imaging using ioflupane (I^{123}) is the most widely utilized tracer to assess DAT binding, both in research studies and clinical practice. Based on the current level of evidence, a quantitative DAT SPECT biomarker, the specific binding ratio in the lowest putamen adjusted for age and sex, is used to categorize individuals as DAT deficit (D+) or without DAT deficit (D-). Standardized DAT acquisition and analysis are essential. Several analysis protocols are utilized for DAT measurement, and a goal will be to harmonize these outcomes to provide a unified field standard. Data from several studies including the Parkinson Progression Markers Initiative (PPMI)[71] and recent clinical trials are available to establish this quantitative standard.

There are also limited but evolving data showing that several PET tracers targeting DAT or VMAT effectively detect dopaminergic dysfunction[62,72,73]. These PET tracers might be an alternative to ioflupane imaging. All PET and SPECT dopamine tracer data can ultimately be harmonized to a single quantitative scale similar to the development of centiloids for amyloid imaging[74].

Importantly, current data strongly suggest that n-asyn can be detected by SAA before dopamine dysfunction is detected by imaging[2,75]. While the overwhelming majority of individuals develop detectable DAT loss prior to functional impairment[65-69], nonetheless, we recognize it is possible that signs/symptoms and functional impairment may occur in S+ individuals in the absence of DAT deficit. Additional data will clarify how frequently and under what conditions this may occur. Further, we acknowledge that presynaptic dopaminergic dysfunction is not specific for NSD[73].

We recognize that NSD is a multisystem disease that may involve neuronal degeneration widely in the central and peripheral nervous system involving non-dopaminergic pathways[76]. With validation of biomarkers that reflect pathology in other systems will come the opportunities to incorporate them into the NSD-ISS.

G status

Genetic variants may either cause or increase risk for NSD**.** Fully penetrant pathogenic variants in *SNCA,* the gene that encodes alpha-synuclein*,* are disease-defining in the NSD-ISS[38]. Individuals with these rare variants have a high certainty of developing n-asyn pathology. Other genetic variants (G) identify individuals who may be at risk (R), but these individuals do not have NSD unless they demonstrate evidence of n-asyn (S+).

Genetics play a pivotal role in understanding NSD biology, genetically-driven disease subtyping, and guiding therapeutic development. Pathogenic variants in numerous genes are associated with NSD[40,77,78]. Approximately 10-15% of clinically-diagnosed PD cases carry pathogenic

variants, most commonly in *GBA1* and *LRRK2*[79]. Pathogenic variants in *GBA1* have also been identified in individuals with a clinical diagnosis of DLB[40]. These variants confer varied risk that is both variant-dependent and increases with age[80]. Some less common genetic variants have high penetrance, such as bi-allelic pathogenic variants in *VPS13C, PARK7, PINK-1, and PRKN*[77]. In addition, over 90 single nucleotide polymorphisms have been combined into a genetic risk score that is associated with increased risk of clinical PD[81]. Given the low or variable penetrance of many genetic traits associated with NSD, and that many individuals with these variants do not go on to develop evidence for n-asyn, the NSD-ISS does not consider genotype (aside from fully penetrant pathogenic variants in *SNCA*) sufficient to define the disease; however, the genetic understanding of disease continues to evolve and we expect the use of genetics in this staging system to also evolve.

The majority of individuals with risk variants who develop dopamine loss and functional impairment also show n-asyn and would meet criteria for NSD. However, a subset of individuals, including some of those with disease-driving *LRRK2* variants or the majority of those with disease caused by pathogenic *PRKN* variants, have evidence of dopaminergic dysfunction (D+) but do not have evidence of n-asyn[2-4,82-84] (S-D+G+). These individuals do not have NSD and must be defined and staged separately (see below).

Overview of the NSD-ISS

NSD is a continuum, but defining discrete stages is required to provide a framework for therapeutic development, from the earliest stages of disease, when pathologic changes are identifiable but there are no clinical manifestations or functional consequences, to advanced disease (Figure 1).

We propose an integrated staging system anchored on the disease biology. The biologic definition of NSD is key to enable staging based on biomarkers in Stages 1 and 2. Stages 3-6 are defined by integrating clinical signs/symptoms and specifically anchors for their functional impact with biomarkers (Table 1, Figure 2).

Individuals with pathogenic variants potentially associated with NSD but who do not have evidence for n-asyn are designated as low or high genetic risk (R^L or R^H respectively). They are referenced here to allow a framework within which to conduct research and clinical trials, but they do not have NSD and thus are not assigned a stage.

The NSD-ISS

Stage 0, the first stage of NSD, is solely defined by the presence of fully penetrant pathogenic variants in *SNCA*. These cases are rare but important for understanding NSD biology and targeted therapeutic development aiming to prevent progression to Stage 1 and beyond.

Stage 1 and beyond require detection of n-asyn (S+). Stage 1A includes individuals with biomarker evidence of n-asyn (S+), without evidence of dopaminergic dysfunction (D-), and no relevant signs/symptoms. Stage 1B includes individuals with biomarker evidence of n-asyn (S+) *and* dopaminergic dysfunction (D+), but no relevant signs/symptoms or functional abnormalities. We separate Stage 1A versus Stage 1B based on limited, but accumulating, data that in the majority of cases n-asyn precedes onset of dopaminergic dysfunction but more confirmatory evidence will be required.

Stage 2 is defined by the presence of subtle clinical signs/symptoms, but without functional impairment. Clinical signs/symptoms may be motor or non-motor. Non-motor signs/symptoms include olfactory dysfunction, dysautonomia (orthostatic hypotension, heart rate abnormalities), constipation, neuropsychiatric symptoms (depression, anxiety), mild cognitive impairment, and disorders of sleep and wakefulness (RBD, excessive daytime sleepiness). Some non-motor symptoms, like anxiety, depression, constipation, may be nonspecific, resulting from processes unrelated to n-asyn that are common in aging. The lack of specificity of these clinical features is mitigated by the requirement for n-asyn in these individuals, but we recognize that there is some uncertainty in the spectrum of the clinical features advancing individuals from NSD Stage 1 to 2.

Similar to Stage 1, Stage 2 is subdivided into 2A and 2B based on presence of biomarkers. Ultimately, progression of biomarkers during Stages 1 and 2 may provide an opportunity to test therapeutics that slow/prevent individuals with evidence of n-asyn from developing dopaminergic dysfunction.

Stage 2B and beyond require presence of both biomarkers of n-asyn (S+) AND dopaminergic dysfunction (D+). The majority of individuals with n-asyn will demonstrate dopaminergic dysfunction at the time of motor, cognitive, or other non-motor functional impairment[59,65-69], but additional evidence is required to determine how often functional impairment may occur in NSD with n-asyn before dopaminergic dysfunction is detected. Current data suggest this is uncommon, but additional data could require modification in the requirement for both (S+) and (D+) to advance to Stage 3.

In Stages 3-6, the severity of functional impairment defines each progressive stage. Functional impairment can be driven by relevant motor, cognitive, or other non-motor clinical signs/symptoms. While this staging system is grounded on the NSD biological framework, defining the degree of functional impairment using a data-driven approach is necessary for therapeutics development and ultimate clinical research applicability. We have conceptualized functional impairment qualitatively as progressing along the continuum of *slight, mild, moderate, severe* and provide categorical descriptors of such (Table1).

Most patients with *newly diagnosed* PD, as defined by current clinical diagnostic criteria[20] will be Stage 3, but some without functional impairment will be Stage 2B. This staging based on biology and functional impairment is a major strength of the proposed staging framework, particularly to guide selection of participants for targeted drug development. Notably, patients with primarily cognitive syndrome and NSD-defining biology will fit in the proposed staging system based on detailed anchors of cognitive functional impairment.

While operational definitions of anchors for functional impairment for Stage 3-6 are beyond the scope of this conceptual paper, they are critical for future versions of the NSD-ISS. We envision that the field will soon align on these as data emerge. Examples of these anchors will be provided in a separate report to begin a data-driven discussion to develop consensus. Specifically, data derived from motor, non-motor, and cognitive functional rating scales assessments (i.e., ability to perform activities of daily living) in prospective cohort studies and clinical trials will be used to define thresholds for Stages 3-6, akin to the HD-ISS[12]. The Movement Disorders Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS)[85] Parts I and II are the most widely used measure of functional impairment in NSD and may be applied as a starting point. However, the MDS-UPDRS has limited sensitivity to detect changes in function in earlier disease stages and likely new scales and approaches need to be developed[86].

Biologically-Defined NSD Encompasses PD, DLB, RBD and other Clinical Syndromes

The core principle of the NSD-ISS is that NSD is defined by biology. Yet, a single disease based on a single biology may have protean clinical manifestations. As such, individuals with NSD may have a range of clinical syndromes (Table 2). In most cases, these syndromes will overlap, and contributions from motor and non-motor features will produce a cumulative impact on functional impairment. The key clinical syndromes arising from NSD are n-asyn-driven motor parkinsonism and cognitive syndromes—currently diagnosed as PD, PDD, or DLB based on clinical diagnostic criteria[20-22]. Other well described syndromes include RBD[87], dysautonomia (including PAF)[88]; neuropsychiatric symptoms may predominate as well[17,19]. While NSD-ISS provides unifying biological definition and staging, recognition of clinical syndromes is important to guide syndrome-focused symptomatic management, family support and education.

NSD is a continuum

An important implication of the biologically-based framework of the NSD-ISS is *that a clinical diagnosis of PD, PDD, or DLB is neither sufficient nor necessary for a NSD diagnosis*. The NSD-ISS unifies what is currently defined as prodromal PD and DLB[17-19] and related disorders along the same continuum of progression of the NSD neurodegenerative process (Figure 1). The current distinction of prodromal versus clinically-diagnosed PD or DLB is artificial, lacks standardized criteria for transition and creates barriers in therapeutic development. The current terminology of "phenoconversion" lacks operational definition and is faulted by subjectivity. There is tremendous interest in enrolling currently-defined "prodromal populations" into trials testing experimental therapeutics targeting specific molecular pathways[6,7]. However, the success of such programs depends on identifying participants with a unifying underlying disease biology and establishing the framework for defining progression. A biologic definition and integrated staging system aim to accomplish both goals.

Incorporating other pathologies into the NSD-ISS framework

Many individuals have mixed pathology and will have other diseases in addition to NSD. A key challenge for the NSD-ISS will be to assess the relative impact of clinical functional impairment (particularly cognitive) in individuals with multiple underlying pathophysiological processes and variable clinical syndromes. A goal will be to incorporate biomarkers of AD pathology and other conditions into NSD staging to better understand the key biologic mechanisms resulting in functional impairment.

Individuals who are not NSD

S-D+G+

There are subsets of individuals with genetic variants (G+) such as in *LRRK2 or PRKN* who have evidence of dopaminergic dysfunction (D+) but do not have evidence of n-asyn (S-)[2-4,82-84,89]. These S-D+G+ individuals do not have NSD (Table 3). Importantly, elucidating this unknown pathology in these individuals[82,90] is a research priority. This biologic heterogeneity is only evident when the disease is defined by its biology and highlights the value of biologic rather than clinical disease definition. Understanding the biology of S-D+G+ will inform and improve clinical trial design. For example, *LRRK2*+ S- participants will be included in *LRRK2*-targeted therapeutic studies independent of their S status[91] but will not be candidates for asyn-targeting therapeutic studies.

S-D+G- with known biology

As discussed, MSA is an alpha-synucleinopathy marked by predominantly glial asyn accumulation[1,29]. The structure and characteristics of pathologic asyn in MSA differ from NSD[30,31];While individuals with MSA demonstrate evidence of pathologic asyn[1], it is distinct from the n-asyn that defines NSD, and importantly is differentiated by current n-asyn specific CSF SAA[30,50,52].

Other individuals with dopamine dysfunction without n-asyn also do not have NSD but may have other known biologies (Table 3). Examples include progressive supranuclear palsy (PSP) and corticobasal degeneration, caused by tau- or TDP-related neurodegeneration[92].

S-D+G- with unknown biology

About 5% of individuals with sporadic PD based on current clinical criteria[2] have dopaminergic dysfunction and functional impairment without n-asyn and without relevant genetic variants or alternative known biology (S-D+G-) (Table 3). These individuals do not meet the biologic definition for NSD. Further studies will elucidate the relevant biologies underling the neurodegenerative process in these individuals. Importantly, these individuals should not be enrolled into asyn-targeting therapeutic trials, again highlighting the importance of accurate biological definition.

NSD-ISS will accelerate clinical trials and therapeutic development

During the process of development of the NSD-ISS, there was broad consensus from key stakeholders[93] that adoption of this staging system would allow the field to accelerate and improve clinical therapeutic development at all disease stages, from prior to onset of signs/symptoms through mild to severe functional impairment. Identifying individuals based on biologic characteristics enables a new approach to developing therapies targeting relevant biology and testing therapeutic strategies that will pave the way for precision medicine in the treatment of NSD[7].

The NSD-ISS further provides a framework and a clearly-defined roadmap for clinical trial design and clinical trial evaluation by key constituencies, including pharmaceutical drug developers, regulators, academic experts and clinical trial participants. The staging provides a consistent and uniformly understood definition of the study sample at each stage. It also enables and encourages the development of stage-dependent outcomes to allow assessment within a stage and to define changes between stages[94] (e.g., outcomes could reflect change from Stage 2B to Stage 3 or ultimately from Stage 1 to Stage 2). A major advantage of the NSD-ISS will be to reduce heterogeneity in clinical trials by requiring biologic consistency within the study sample rather than the current clinical requirements for early PD or DLB that may have wide variability.

Future Directions

Gaps in Knowledge and Limitations of the NSD-ISS

While current data strongly support the biologic definition for NSD and the NSD-ISS, we recognize that there are gaps in knowledge and that our understanding of NSD biology and staging will evolve. These gaps and limitations in turn define key research priorities for the field to inform future iterations of the staging system as outlined below.

- The S and D domains are currently categorical. There is a crucial need for quantitative biomarkers to measure disease onset, progression, and response to therapy. Equally

important is to develop biomarker platforms that are feasible and cost effective for inclusive population screening.

- The timeline of evolution along the axis of biological staging is unknown. Such data will be essential to enable therapeutic development for true disease prevention.
- Understanding the biology of individuals who do not have NSD is a high priority.
- As the field evolves, and biomarkers emerge that reflect molecular changes of underlying mechanisms of neurodegeneration (i.e., mitochondrial, lysosomal, inflammatory and other pathways[1]) that are NSD-specific, additional biologic anchors will be introduced into the staging system.
- NSD is a multisystem disease that involves neurodegeneration in other neurotransmitter systems besides dopamine[76], and with validation of biomarkers that reflect pathology in these systems will come the opportunities to incorporate them.
- Defining specific functional anchors across stages 3-6 was outside of the scope of this conceptual paper but is underway. Several observational cohort studies, including the PPMI[71], DLB consortium[95], and clinical trials[96] offer critical data that will allow validation of such anchors[97].
- A key challenge for the NSD-ISS will be to assess proportional impact of multiple co-pathologies on clinical functional impairment (particularly cognitive impairment). Ultimately acquiring AD and other neurodegenerative biomarkers may become a routine component of NSD staging.

Conclusion

We propose a biological definition and integrated staging system for NSD. The current definition of neurodegenerative illnesses as traditional clinically-defined syndromes limits our understanding of the biology of neurodegeneration and slows therapeutic development, especially targeting individuals before signs/symptoms emerge. The strength of this integrated biologic staging system is that it is based on a biological definition of disease and integrates biology and progressive functional impairment. Starting with homogeneous biology enables both the investigation of all clinical syndromes encompassed by NSD and encourages the development of stage-specific outcomes to further assess disease progression and therapeutic intervention. The NSD-ISS will enhance trial efficiency, provide a consistent and uniformly understood definition for the study sample at each stage, and enable selection of appropriate trial endpoints. NSD-ISS will evolve as new data and new biomarkers emerge.

Tables and Figures

Figure 1: **Hypothetical framework of NSD integrated staging system**

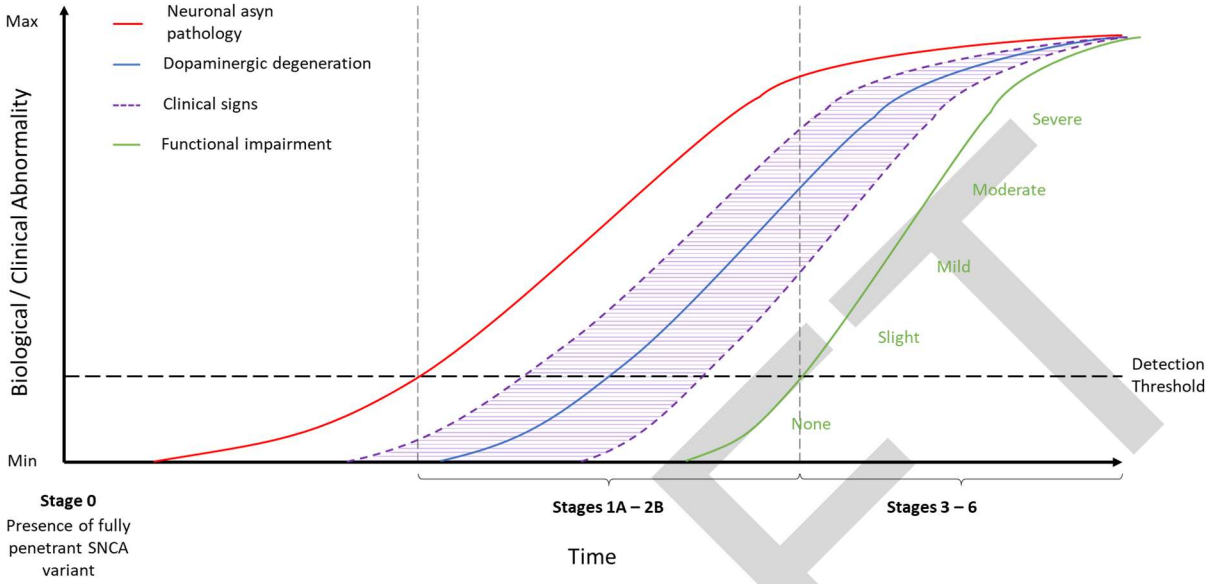
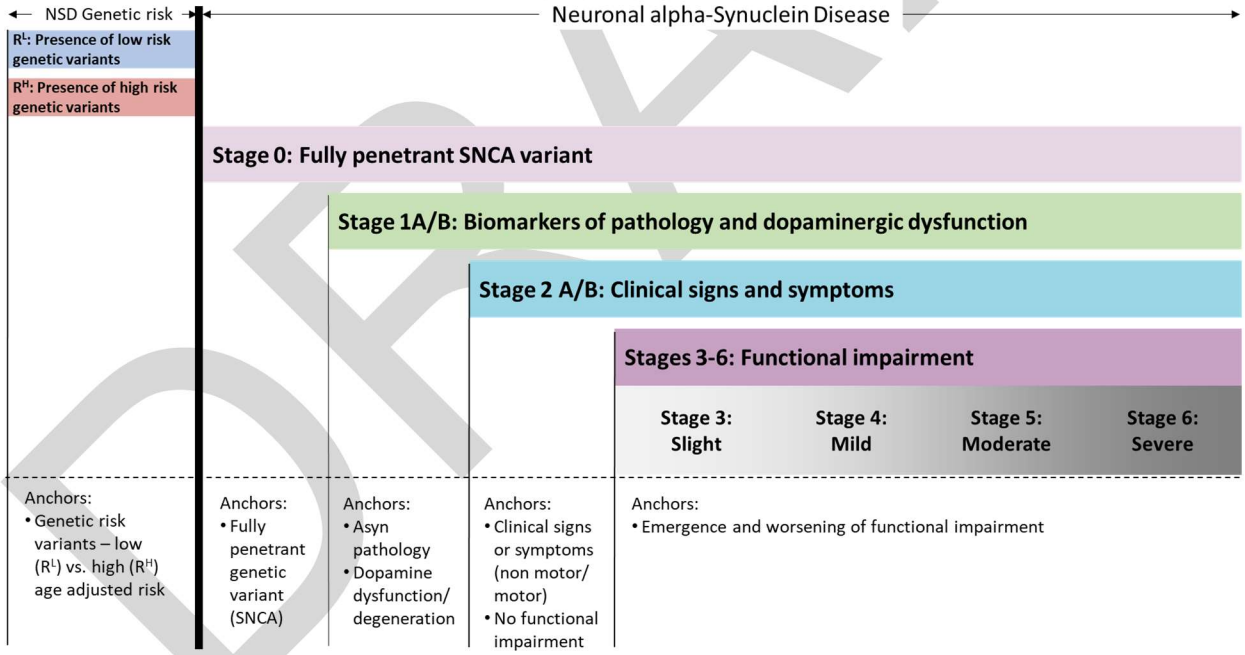


Figure 2: **NSD: an integrated staging system**



478 ***Table 1:*** **NSD-ISS: an integrated staging system**

Disease Conti-nuum	Stage	Stage Definition	aSyn Bio-marker (S)	Dopamine Dysfunction Biomarker (D)	Clinical Signs and Symptoms Attributable to PD, DLB and other NSD syndromes	Functional Impairment Attributable to PD, DLB and other NSD syndromes
Genetic risk	R^L	(G) Genetic risk variants – Low age adjusted risk (constantly redefined).	-	-	No clinical signs or symptoms	No functional impairment
Genetic risk	R^H	(G) Genetic risk variants – High age adjusted risk (constantly redefined)	-	-	No clinical signs or symptoms	No functional impairment
NSD	0	(G) Fully penetrant *SNCA* variant	-	-	No clinical signs or symptoms	No functional impairment
NSD	1A	Characteristic pathological changes but no evidence of clinical signs/ symptoms	+	-	No clinical signs or symptoms	No functional impairment
NSD	1B	Characteristic pathologic changes plus dopaminergic dysfunction but no evidence of clinical signs/ symptoms	+	+	No clinical signs or symptoms	No functional impairment
NSD	2A	Characteristic pathological changes and subtle detectable clinical signs and symptoms but no functional impairment.	+	-	Subtle clinical signs or symptoms. Can be motor or non-motor: hyposmia, RBD, cognitive abnormalities, constipation, dysautonomia, depression, anxiety.	No functional impairment
NSD	2B	Characteristic pathologic changes plus dopaminergic dysfunction and subtle detectable clinical signs and symptoms but no functional impairment.	+	+	*Same as Stage 2A*	No functional impairment
NSD	3	Characteristic pathologic changes plus dopaminergic dysfunction and clinical signs and symptoms causing slight functional impairment.	+	+	*Relevant* motor and non-motor signs and symptoms increasing in severity, but stage is defined by the degree of functional impairment	Slight: Functional impairment not severe enough to cause uncompensated impairment in complex tasks of daily life and usual activities, such as: finances, transportation, food, household, conversation
NSD	4	Characteristic pathologic changes plus dopaminergic dysfunction and clinical	+	+	*See entry for Stage 3*	Mild: Functional impairment severe enough to cause some uncompensated

		signs and symptoms causing mild functional impairment.				impairment in complex tasks of daily life and usual activities, but basic tasks of daily life related to personal care are intact, such as: bathing, dressing, walking, using the toilet, eating
NSD	5	Characteristic pathologic changes plus dopaminergic dysfunction and clinical signs and symptoms causing moderate functional impairment.	+	+	*See entry for Stage 3*	Moderate: Functional impairment severe enough to require assistance with basic tasks of daily life
NSD	6	Characteristic pathologic changes plus dopaminergic dysfunction and clinical signs and symptoms causing severe functional impairment	+	+	*See entry for Stage 3*	Severe: Functional impairment severe enough to require dependence on others for basic tasks of daily life

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Table 2: **NSD diagnosis: spectrum of clinical syndromes**

NSD diagnosis	NSD clinical syndromes*
NSD is defined by biology: Clinical entities represent a spectrum of the clinical syndromes under the biological of NSD	NSD. Parkinson's syndrome (Motor syndrome)
	NSD. DLB syndrome (Cognitive syndrome)
	NSD. Neuropsychiatric syndrome (i.e., disease-relevant anxiety, depression)
	NSD. RBD syndrome
	NSD. Other non-motor syndromes (i.e., autonomic, hyposmia)

480 *Majority of individuals will have a combination of multiple and overlapping syndromes but may
481 have initial clinical presentation within a particular domain

Table 3: **NSD and not NSD**

Biomarker Profile			Designation
S	D	G	
+	+/-	+/-	NSD (neuronal synuclein disease) NSD can have comorbid pathology (i.e., A+/T+)
-	+	+	Genetic variants with dopaminergic dysfunction but without evidence of n-asyn
-	+	NA	Dopaminergic dysfunction with motor or cognitive functional impairment with predominantly glial asyn (i.e., MSA)
-	+	NA	Dopaminergic dysfunction with motor or cognitive functional impairment without n-asyn-known biology (i.e., PSP, CBD)
-	+	-	Dopaminergic dysfunction with motor or cognitive functional impairment without n-asyn and without known genetic variant-unknown biology
-	-	-	Individuals with motor or cognitive functional impairment but without n-asyn, dopaminergic dysfunction, known biology or relevant genetic variants

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