# Biomarkers in Medicine

# Parkinson's disease biomarkers: perspective from the NINDS Parkinson's Disease Biomarkers Program

Biomarkers for Parkinson's disease (PD) diagnosis, prognostication and clinical trial cohort selection are an urgent need. While many promising markers have been discovered through the National Institute of Neurological Disorders and Stroke Parkinson's Disease Biomarker Program (PDBP) and other mechanisms, no single PD marker or set of markers are ready for clinical use. Here we discuss the current state of biomarker discovery for platforms relevant to PDBP. We discuss the role of the PDBP in PD biomarker identification and present guidelines to facilitate their development. These guidelines include: harmonizing procedures for biofluid acquisition and clinical assessments, replication of the most promising biomarkers, support and encouragement of publications that report negative findings, longitudinal follow-up of current cohorts including the PDBP, testing of wearable technologies to capture readouts between study visits and development of recently diagnosed (*de novo*) cohorts to foster identification of the earliest markers of disease onset.

Katrina Gwinn[1], Karen K David[‡,1], Christine Swanson-Fischer[‡,1], Roger Albin[2], Coryse St Hillaire-Clarke[3], Beth-Anne Sieber[1], Codrin Lungu[1], F DuBois Bowman[4], Roy N Alcalay[5], Debra Babcock[1], Ted M Dawson[6], Richard B Dewey Jr[7], Tatiana Foroud[8], Dwight German[9], Xuemei Huang[10], Vlad Petyuk[11], Judith A Potashkin[12], Rachel Saunders-Pullman[13], Margaret Sutherland[1], David R Walt[14], Andrew B West[15], Jing Zhang[16], Alice Chen-Plotkin[17], Clemens R Scherzer[18], David E Vaillancourt[19] & Liana S Rosenthal*[20]

*Author for correspondence:
liana.rosenthal@jhmi.edu
‡Authors contributed equally

For author affiliations, please see the end of this article.

## Potential role of biomarkers in the diagnosis & management of Parkinson's disease

There has been significant progress in our understanding of the biology of Parkinson's disease (PD); however, current therapies treat only the symptoms of PD. Identification of neuroprotective agents to slow or halt disease progression is, therefore, an urgent need. Several Phase III studies testing putative neuroprotective agents in PD failed for a number of reasons [1,2]. First, due to the indolent nature of PD, the typical time from diagnosis to death is 6.9–14.3 years [3] and the varying rate of decline makes it difficult to design neuroprotective trials. Second, PD is heterogeneous and includes a small but significant fraction of PD mimics (parkinsonism). The fact that PD is itself a syndrome composed of a variety of overlapping disorders with variable natural histories further complicates

heterogeneity. Moreover, co-morbid conditions likely influence disease expression [4]. Finally, evaluations based on commonly used clinical research instruments (limited by inter-rater reliability and practice effects) are affected by both symptomatic treatments and co-morbid conditions. The most widely used clinical instrument in PD neuroprotection trials is the Movement Disorder Society-Unified Parkinson's Disease Rating Scale, yet this scale is constructed largely to measure dopaminergic therapy responsive features of PD, not the treatment resistant aspects that are characteristic of advanced PD [5]. As a result, changes in dopaminergic treatment during a neuroprotection trial could confound the assessment of disease progression (as measured by this scale) or have to be prohibited by the protocol, thus greatly limiting subjects who can participate in the study. There are no specific biomarkers for any stage

in PD and the identification of specific PD biomarkers would be a major advance in implementing effective clinical trials for possible neuroprotective treatments. Biomarkers would allow better subject selection and stratification, evaluation of disease activity and target engagement, leading to improved aims in clinical trials. It is unlikely that any single biomarker will satisfy all needs for higher quality PD neuroprotection trials. Therefore, a plurality of approaches aimed at realizing several different and complementary types of PD biomarkers is needed.

A number of efforts are underway toward identification and validation of PD biomarkers (Table 1). One such effort is the National Institute of Neurological Disorders and Stroke (NINDS) Parkinson's Disease Biomarkers Program (PDBP). Prior to establishing the PDBP, NINDS staff conducted a workshop in 2012 surveying the biomarkers landscape and relevant NINDS programs. These discussions included stakeholders and experts in the field, as well as others from the scientific community, in order to obtain broad input regarding the appropriate next steps [6]. The US FDA perspective on biomarkers validation was used to consider progress and to identify possible gaps, as well as to calibrate the activities of the program with the long-term goal of moving biomarkers toward use in PD neuroprotective and other PD clinical trials. As a result of this process, it was determined that the PDBP would encompass creation of three broad components needed for biomarkers research: a data management resource (DMR) to support standardization and data sharing; well-characterized longitudinal clinical cohorts, with detailed clinical data collected and biospecimens banked; and laboratory and clinically-based biomarker discovery [7]. PDBP was also designed to fill the gap between two existing PD biomarkers programs established by the Michael J Fox Foundation: the Parkinson's Progression Marker Initiative (PPMI), whose goal is validation of biomarker discovery projects [8] and BioFIND, an observational cross-sectional study cohort [9]. The latter does not include hypothesis-based or discovery research. PDBP fills the gap between these two efforts and complements both by: creating a longitudinal data and sample resource that includes a broader clinical spectrum of PD and parkinsonism than other collections; funding a range of biomarkers discovery projects; and creating a resource for replication of early discoveries. Unlike PPMI, PDBP is agnostic regarding results of dopamine transporter single-photon emission-computed tomography (SPECT) imaging, presenting phenotype (tremor predominant or postural instability and gait difficulty [PIGD]) or disease duration, or stage. Most PD subjects included are taking dopaminergic drugs that are documented

both with respect to drug identity and dose at the time of each study visit. This information enables calculation of the levodopa equivalent daily dose for each PD subject at each visit, which can be used to correlate with any putative biomarkers discovered as part of the project. The data collected in PDBP includes a standard set of assessments that would be collected on a typical subject in a future clinical trial [10]. PDBP also complements another effort, the Harvard Biomarker Study (HBS), a longitudinal case-control study which developed a biobank of specimens aimed at biomarker discovery.

Biomarkers can be categorized in terms of context of use (defined in Table 2) [11]. The PDBP has projects addressing many purposes, including susceptibility/risk ('trait') biomarkers, diagnostic ('state') biomarkers, disease progression ('rate') biomarkers, prognostic biomarkers and predictive biomarkers (see Table 2). Monitoring, pharmacodynamic and safety biomarkers (also defined in Table 2) are used in relationship to a given therapeutic; these biomarker types are usually advanced in concert with the development of neuroprotective and symptomatic treatment agents and, therefore, are not within the scope of PDBP.

Another useful categorization scheme relates to the stage of biomarker development. In this scheme, biomarker efforts can be categorized as being in the discovery, replication/validation or qualification phase.

### Discovery

Discovery in biomarkers research generates new knowledge, drives innovation and provides the input for biomarkers pipeline development. Methods of discovery may include physiological/clinical assessments, genomic/proteomic/metabolomic/RNA methodologies and imaging approaches. Some of these approaches derive directly from basic research (Figure 1). Application of basic research in biomarker development capitalizes on the strengths of the NIH process. This is consequently an appropriate space for NINDS to play a role in filling the gaps in PD biomarkers research. NINDS established the PDBP based on the concept that biomarkers discovery and replication are needed prior to validation and application in clinical trials.

### Replication & validation

Replication and validation are essential components of any scientific process, as biomarkers will only be useful if generalizable and reliable. However, validity and generalizability criteria are not well delineated in the biomarker field. Replication is not a simple or straightforward designation. Replication can mean consistent results obtained in the same laboratory with the same samples; results replicated in the same laboratory with

**Table 1. Multisite Parkinson's disease biomarkers biospecimen and data resources.**

| Project name | Number of cases/controls to date[†] | Original goals (established) | Current goals | Highlighted population features | Sample types shared | Available for study type |
|---|---|---|---|---|---|---|
| NINDS Parkinson's Disease Biomarkers Program | 825 PD/524 controls/44 PSP/4 CBD/28 MSA | Observational, longitudinal as well as hypothesis testing (2012) | PDD and LBD; PSP, CBD, MSA; genetic cohorts; ethnic diversity | Typical cases included to create a 'real world' scenario | Whole blood, plasma, DNA, RNA, CSF, MRI | Discovery, replication, validation |
| Progressive Parkinson's Disease Biomarkers Program | 426 *de novo*[‡] PD/196 controls/64 SWEDD | Observational, longitudinal, *de novo*[‡] and controls (2010) | Genetic cohort (mutation in LRRK2, GBA or SNCA) | *De novo*[‡] DATscan required for inclusion | Whole blood, plasma, serum, urine, DNA, RNA, CSF, MRI and fibroblasts and iPSCs in some of the cohort | Validation |
| Harvard Biomarkers Study | 719 PD (incl. 57 *de novo* PD)/892 HC/641 DC with mild cognitive impairment/150 with AD/101 with other NDD | Observational, longitudinal cohort of the most common movement and memory disorders, and controls (2008) | Lewy body dementias, long-term follow-up, brain donations, multiomics phenotyping | Large sample to reflect heterogeneity within PD; spectrum of neuropathologically interwoven diseases with Lewy body, β-amyloid and tau neuropathology | Whole blood, pellet, plasma, serum, DNA, RNA, miRNA, lymphoblastoid cell line, subset with CSF | Discovery, replication, validation |
| BioFIND | 126 PD/106 controls (recruitment complete) | Observational, cross-sectional (2012) | N/A (completed 2015) | Can be on treatment. Any Hoehn and Yahr Stage. DATscan required for inclusion | Whole blood, pellet, plasma, saliva, urine, DNA, RNA, CSF | Discovery |

†Subjects recruited may not all have all biological sample types.
‡*De novo* indicates a subject with a clinical diagnosis of PD for 2 years or less, not taking PD medications.
CSF: Cerebrospinal fluid; DATscan: A radiopharmaceutical indicated for striatal dopamine transporter visualization using single-photon emission–computed tomography (SPECT) brain imaging to assist in the evaluation of adult patients with suspected Parkinsonian syndrome; DC: Disease control; GBA: Glucocerebrosidase gene (glucosylceramidase β); HC: Healthy control; LBD: Lewy body dementia(s); MSA: Multiple system atrophy; NDD: Neurodegenerative disease control; PD: Parkinson's disease; PDD: Parkinson's disease dementia; PSP: Progressive supranuclear palsy; CBD: Corticobasal degeneration; SNCA: α-Synuclein; SWEDD: Subject without evidence of dopamine deficiency (clinically have PD).

different samples; or results replicated in a different setting with the same samples. In addition to replication, analytic and clinical validation are necessary for moving any biomarker forward. Analytic validation is generally applied to studies that establish that the performance characteristics of the test are acceptable in terms of its sensitivity, specificity, accuracy and precision, as applicable [13]. Clinical validation is defined as establishing that the candidate biomarker acceptably identifies, measures or predicts the clinical, biological, physical or state of interest [13]. Thus to meet the requirements for replicating and validating robust biomarkers, the PDBP is a milestone-driven program, in which go/no-go decision points are specified for all discovery and replication projects. In addition, standard blood-based laboratory tests are performed on all PDBP participants and lab results are captured in the PDBP DMR. Quality control of plasma, serum and cerebrospinal fluid (CSF) by the NINDS BioSEND biorepository includes hemoglobin assessment, while the RNA Integrity Number, 260/280 and 260/230 ratios are used to determine RNA and DNA quality, respectively. NIH also sponsored a funding announcement that supported the use of PDBP biosamples for discovery or replication of promising PD biomarkers

defined by specificity, selectivity, accuracy and precision criteria. Study design required the identification of independent discovery and replication cohorts and the use of pooled samples for standardization across laboratories and platforms.

## Qualification

Qualification is a term that applies largely to a regulatory process and designation. For instance, considerations for biomarker qualification at the FDA include: the context of use for drug development, the relationship of the biomarker to clinical outcomes and treatment, assay considerations (including variability), biological rationale for use, strength of association of the biomarker with the clinical state, reproducibility of data and strength of evidence. Note that qualification is not required for biomarker use in a clinical trial prior to submitting biomarker trial data to the FDA for review [14].

## PDBP achievements

The overall philosophy for establishing the NINDS PDBP was to foster scientific breakthroughs and progress, as well as resources, and to facilitate integration of the existing and future projects into a dovetailed

| Table 2. Definitions of biomarker types. | | | |
|---|---|---|---|
| **Type of biomarker** | **Definition based on use** | **Example** | **PD-specific examples** |
| Susceptibility/risk (trait biomarker) | Indicates the potential for developing a condition in an unaffected individual | *BRCA1/2* mutations identify individuals with a predisposition to developing breast cancer | Nonmotor symptoms which occur before motor signs are evident |
| Diagnostic (state biomarker) | Identify individuals with the disease or condition of interest or to define a subset of the disease | Sweat chloride levels used to confirm cystic fibrosis | Tools that differentiate PD from parkinsonism |
| Prognostic | Identify likelihood of a clinical event, disease recurrence or progression | Chromosome 17p deletions assess likelihood of death in chronic lymphocytic leukemia | Cognitive dysfunction in PD higher among individuals who had amyloid and tau pathology |
| Predictive | Identify individuals more likely to experience a favorable or unfavorable effect from a specific intervention or exposure | Potassium channel mutations evaluate children with diabetes to determine benefit from sulfonylurea treatment | Biomarkers to predict the risk of dyskinesias due to PD symptomatic treatment |
| Monitoring (rate biomarker) | Measured serially and used to detect a change in the degree or extent of disease; may also be used to indicate toxicity/assess safety | International normalized ratio used for monitoring patients on warfarin | No current examples in PD treatment |
| Pharmacodynamic/ response | Demonstrate that a biological response has occurred in an individual receiving an intervention or exposure | HIV viral load used when evaluating response to antiretroviral treatment | No current examples in PD treatment |
| Safety | Indicate the presence or extent of toxicity related to an intervention or exposure | Serum creatinine monitors for nephrotoxicity of certain treatments | Monitoring effectiveness and side effects of levodopa |
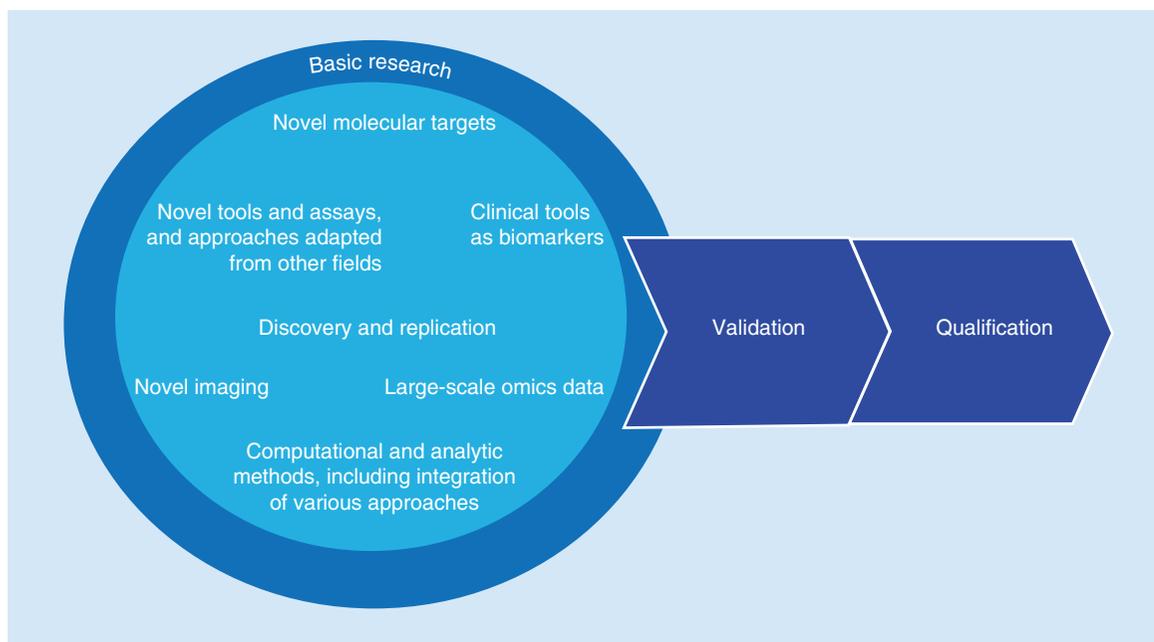
**Figure 1. The typical biomarker development pipeline.** The typical pipeline includes significant basic research in association with well-characterized clinical cohorts. Any biomarkers discovered and replicated, must be validated and shown to qualify as biomarkers.
Reproduced from [12].

sequence from discovery to qualification for application of PD biomarkers in Phase II or III clinical trials. The PDBP concrete goals established in 2012, based on stakeholder and outside expert input, as noted above, were data management, clinical cohorts with standardized biospecimen collection and laboratory-based discovery science [7].

## Data management

The PDBP DMR is the central hub for coordinating data-related activities integral to PDBP and includes seven modules that enable data entry, quality control, data access, data query, biosample access and account management. The DMR contains a common data dictionary used across multiple studies and built upon NINDS common and PD-disease specific data elements [15]. Several unique features associated with various modules of the DMR include ProFoRMS, use of a Global Unique Identifier (GUID) and the Query tool. The ProFoRMS module is an electronic data entry system, which also enables patient scheduling and quality assessment of data prior to uploading to the data repository. The GUID, is a software application that generates a unique identifier for each study participant. This software application uses a subject's personal identifiable information to create a one-way hash code made up of a prefix, an alphanumeric sequence and a check character. The DMR database contains only deidentified data, so the GUID is never directly associated with the personal identifiable information. The

GUID enables linking of all data (genetic, imaging, clinical and biomarker) for an individual subject both within and across studies, and it is the identifier used to join subject level data in the Query tool. The Query tool enables a researcher to select data based on either a form, a data element, or through the use of a defined query. In the Query tool, selected data can be joined (3-way), processed at an individual level and further filtered based on data elements and permissible value ranges. Data can be downloaded either from data generated from queries or directly from studies listed in the data repository. To date, there are have been over 4.7 million records and 400,000 datasets accessed through the DMR query tool. These data have contributed to biomarker discovery [16–18] and disease modeling efforts [19].

## Clinical cohort & biospecimen collection

A total of 1547 individuals have been enrolled in the PDBP studies to date (Table 3). Out of this, 825 individuals have PD, 44 individuals have Progressive Supranuclear Palsy (PSP), 28 individuals are diagnosed with Multiple System Atrophy (MSA), 36 individuals have atypical PD, 23 individuals with Essential Tremor and 525 are control subjects. Twenty-five percent of PDBP participants have a family history of PD and 184 participants were diagnosed with PD within 1 year of PDBP study enrollment. Clinical assessment tools overlap with those used in subject ascertainment in PPMI [8] and BioFIND [9].

**Table 3. Parkinson's Disease Biomarker Program project categorization summary.**

| Investigator | Biomarkers purpose | Project title | Scientific field | Subfield (focus) | Research funding announcement |
|---|---|---|---|---|---|
| Albin | Diagnostic | Serotonin and amyloidopathy | Imaging | PET imaging, serotonergic innverative and a-beta deposition, PD versus aging | Research project grant (Parent R01)† |
| Alcalay | Prognostic | The role of glucocerebrosidase in Parkinson's disease | Genome and transcriptome | Risk assessment PD in GBA mutation carriers | Independent Scientist Award (Parent K02)† |
| Ascherio | Susceptibility/risk | Metabolomics and risk of Parkinson's disease | Proteome and metabolome | Lifestyle and risk for PD | Research project grant (Parent R01)† |
| Bowman | Prognostic | Analytic methods for determining multimodal biomarkers for Parkinson's disease | Multimodal (clinical, imaging) | Statisical techniques to differentiate PD from controls, AD | Exploratory laboratory and analysis projects in Parkinson's disease biomarkers (U18) |
| Chen-Plotkin | Prognostic, susceptibility/risk | Unbiased approaches to novel biomarker discovery in Parkinson's disease | Proteome and metabolome | Plasma-based markers for cognitive performance/risk | Studies in Parkinson's disease biomarkers discovery (U01) |
| Dawson; Rosenthal | Resource creation, subject characterization | Johns Hopkins medicine biomarker discovery in Parkinson's disease | Clinical | Cohort assessment and collection | Studies in Parkinson's disease biomarkers discovery (U01) |
| Dewey | Resource creation, subject characterization | Diagnostic and prognostic biomarkers for Parkinson's disease | Clinical | Cohort assessment and collection, gait and balance as rate biomarkers | Studies in Parkinson's disease biomarkers discovery (U01) |
| German | Diagnostic | Diagnostic and prognostic biomarkers for Parkinson's disease | Proteome and Metabolome | Peptoid identification to differentiate PD vs AD, PD vs controls | Studies in Parkinson's disease biomarkers discovery (U01) |
| Huang | Diagnostic, prognostic, progression | Multimodal MRI markers of nigrostriatal pathology in Parkinson's disease | Imaging | DTI and MRI measures of iron-related proteins; PD vs PDism | Studies in Parkinson's disease biomarkers discovery (U01) |
| Petyuk | Diagnostic | Development of Lewy bodies biofluid signatures by targeted proteomics | Proteome and metabolome | Identify proteins in pathologically confirmed LB cases; validate in PD/control blood-based sample | Exploratory laboratory and analysis projects in Parkinson's disease biomarkers (U18) |
| Potashkin | Diagnostic | Blood RNA biomarkers of Parkinsons disease and progressive supranuclear palsy | Genome and transcriptome | Identify an RNA signature that differentiates PD from PSP | Parkinson's Disease Biomarker Program discovery projects (U01) |
| Saunders–Pullman | Diagnostic, progression | Evaluation of glucocerebrosidase pathway biomarkers in Parkinson disease | Proteome and metabolome | Evaluate GBA pathway markers in GBA-PD, IPD, controls and their clinical correlates | Parkinson's Disease Biomarker Program discovery projects (U01) |

Comment: the last column illustrates that majority of the projects in the Parkinson's disease biomarkers discovery are because of initiatives coordinated by program officers.
†Unsolicited projects.
AD: Alzheimer's disease; ET: Essential tremor; GBA: Glucocerebrosidase; IPD : Idiopathic Parkinson's disease; PD: Parkinson's disease; PDism: Non-PD Parkinsonism; PET: Positron emission tomography; PSP: Progressive supranuclear palsy.

## Table 3. Parkinson's Disease Biomarker Program project categorization summary (cont.).

| Investigator | Biomarkers purpose | Project title | Scientific field | Subfield (focus) | Research funding announcement |
|---|---|---|---|---|---|
| Scherzer | Diagnostic | Biomarkers for early intervention in Parkinson disease | Genome and transcriptome | Regulatory RNA studies of PD pathogenesis (neuropathology), blood and spinal fluid of PD cases | Studies in Parkinson's disease biomarkers discovery (U01) |
| Scherzer | Prognostic | Parkinson disease: predicting the future | Genome and transcriptome | Exome sequencing to analyze variants that disrupt susceptibility and familial PD genes | Parkinson's Disease Biomarker Program discovery projects (U01) |
| Vaillancourt | Diagnostic, prognostic | Noninvasive markers of neurogeneration in movement disorders | Imaging | DTI to differentiate PD from PDism, ET and controls and measure progression | Biomarkers discovery in Parkinsonism (U01) |
| Vaillancourt; Corcos | Diagnostic, prognostic | Neuroimaging biomarkers in Parkinsonism: differentiating subtypes and tracking disease progression | Imaging | Using free-water and task-fMRI to compare PD, PDism and controls | Exclude this? Please see comment |
| Walt | Diagnostic | Detection of post-translationally modified proteins as a biomarker panel for Parkinson's disease | Genome and transcriptome | Use SiMoA assays to detect and quantify post-translationally modified proteins, to compare PD, PDism and controls | Parkinson's Disease Biomarker Program discovery projects (U01) |
| West | Prognostic | LRRK2 and other novel exosome proteins in Parkinsons disease | Proteome and metabolome | Determine if urinary exosome proteins are associated with PD susceptibility and progression | Exploratory laboratory and analysis projects in Parkinson's disease biomarkers (U18) |
| West | Prognostic | Exosome LRRK2 in predicting Parkinson disease phenotypes | Proteome and metabolome | Determine if pS(1292)-LRRK2 levels can predict the risk of PD in LRRK2 carriers, and if it can predict progression in IPD | Parkinson's Disease Biomarker Program discovery projects (U01) |
| Zhang | Diagnostic | Large-scale biomarker discovery and validation for Parkinson's disease | Proteome and metabolome | Use protein and peptide-based approaches to discovery, replicate and validate markers to differentiate PD from controls | Studies in Parkinson's disease biomarkers discovery (U01) |

Comment: the last column illustrates that majority of the projects in the Parkinson's disease biomarkers discovery are because of initiatives coordinated by program officers.
†Unsolicited projects.
AD: Alzheimer's disease; ET: Essential tremor; GBA: Glucocerebrosidase; IPD: Idiopathic Parkinson's disease; PD: Parkinson's disease; PDism: Non-PD Parkinsonism; PET: Positron emission tomography; PSP: Progressive supranuclear palsy.

The PDBP has collected samples from 218 individuals with at least a 2-year follow-up to date. Biospecimens obtained on all subjects include DNA, RNA, plasma, serum, whole blood and many subjects also have provided CSF. These samples were collected in a standardized fashion across sites, utilizing comparable protocols as used in the Alzheimer's Disease Neuroimaging Initiative [20], PPMI [8] and BioFIND [9]. Samples are processed and stored at the BioSEND repository at Indiana University using standard operating procedures. All specimens undergo uniform quality assessment at BioSEND upon receipt. Biosample requests are submitted through an online application form [21] and reviewed by the PD Biospecimen Resource Access Committee (BRAC) established by NINDS. The PD BRAC also reviews requests for biosamples from BioFIND, the Michael J Fox Foundation LRRK2 cohort and the HBS through this online system, thus enabling a researcher to request samples from multiple cohorts in a single application process. Once approved, biospecimens are distributed from BioSEND in a systematic manner [22].

PDBP clinical data are made available via a controlled process which requires the establishment of an account with the DMR. Requirements for account access include agreements to: not share the data with third parties; to not seek to identify any individual participating in the study; and acknowledge the PDBP in resulting publications. Once the PD BRAC has approved biosample access, the PDBP DMR Query tool enables an investigator to link clinical data to biosample availability through the GUID. PDBP biosamples selected by the investigator are processed through the DMR Order Manager. The DMR Order Manager provides a unique tool for linking biorepository and data management functions. To date, a total of 24 PDBP biosample requests have been received and 5228 biosamples have been distributed. A total of 967 DNA samples have been used for genotyping with the NeuroX chip [19,23]. NeuroX data are available through the DMR, once a DMR account holder has completed the PDBP Genomic Data Use Certificate. A total of 1308 DNA samples are currently being used for whole genome sequencing analysis and data from this analysis will be available through the DMR in 2017. Eight hundred and thirteen DNA samples have been distributed for targeted sequencing of validated PD risk alleles and data will be available through the DMR in 2018. One hundred and thirty-eight RNA samples have been distributed for RNA biomarker replication and data will be available in the DMR in 2018. One thousand five hundred and thirty-one plasma samples are currently being analyzed for urate and vitamin D levels. This data

will be available in the DMR in 2017. A complete list of PD BRAC-approved biomarker discovery projects are provided in Supplementary Table 1.

## Clinical & laboratory-based discovery science

A typical biomarker 'pipeline' is represented in Figure 1. Discovery science is an essential part of the biomarkers pipeline. While it is represented commonly as a uniform enterprise in most biomarkers research discussions, biomarker discovery research is complex, highly textured and not monolithic as often represented in biomarkers literature; the latter typically delves more deeply into clinical trial requirements and the US FDA qualification process. A challenge which warrants further evaluation is how the biomarker discovery approaches will interact with each other as well as with the other components of the biomarker qualification process.

## Overview of promising biomarkers

The PDBP Steering Committee (consisting of PDBP Principal Investigators and NINDS staff), along with academic and industry experts, convened in August 2016 to discuss the present landscape of PD biomarkers in general and to make recommendations for the PD biomarker field overall. The PD biomarker field was considered in the following categories to facilitate discussion: Clinical and Physiological biomarkers, Imaging biomarkers, Genomic and Transcriptomic biomarkers and Proteomic and Metabolomic biomarkers.

## Clinical biomarkers

We consider a clinical biomarker to be an objectively measured assessment that characterizes specific traits of individuals with PD. Examples include gait measurements, smell testing, cognition and neuropsychiatric assessments. Some of these candidate biomarkers overlap with clinical end points for other biomarker investigations. It is not entirely clear how to differentiate a clinical biomarker *per se* from well-validated clinical assessment tools. The reality is that several putative biomarkers are also clinical assessment tools. Well-validated clinical assessments tools that are considered likely to be useful as biomarkers are shown in Table 4.

Clinical biomarkers may be useful to measure disease activity and progression. Clinical measures, in particular, the Unified Parkinson's Disease Rating Scale (and the more recently updated Movement Disorder Society-Unified Parkinson's Disease Rating Scale) have long been used as outcome measures in clinical trials in PD [29]. There are a number of major limitations to using this scale for disease modification research in PD including: the scale objectively mea-

| Table 4. Clinical biomarkers. | | | | | |
|---|---|---|---|---|---|
| **Clinical finding** | **Assessment** | **Potential uses** | **Limitations** | **Study (year)** | **Ref.** |
| Hyposmia | UPSIT | Enrichment of presymptomatic cohort; may be useful for PD risk | Not specific to PD | Xiao et al. (2014) Nalls et al. (2015) | [24], [25] |
| RBD | RBD screening questionnaire | Enrichment of presymptomatic cohort; may be useful for PD risk score | Not specific to PD, long delay between RBD symptoms and PD symptoms. comparatively low sensitivity/specificity | Iranzo et al. (2006) | [26] |
| | Polysomnogram | Enrichment of presymptomatic cohort; may be useful for PD risk score | Not specific to PD, long delay between RBD symptoms and PD symptoms | Iranzo et al. (2006) | [26] |
| Motor symptoms | UKBB | Diagnostic criteria | Approximately 10% misdiagnosis; not progression marker, some criteria need revision† | Hughes et al. (1992) | [27] |
| | MDS diagnostic criteria | Updated diagnostic criteria | Not progression marker | Postuma et al. (2015) | [28] |
| | MDS-UPDRS III | Progression, evaluation of change after therapeutic intervention | Scores vary with levodopa-induced motor fluctuations and amount | Goetz et al. (2008) | [29] |
| Cognitive | MoCA | Global cognitive screening; diagnostic for cognitive changes; monitoring progression | Minimal testing per domain; not specific to PD | Dalrymple-Alford (2010) | [30] |
| | SCOPA-COG | Global cognitive screening; diagnostic for cognitive changes; monitoring progression | Minimal testing per domain | Marinus et al. (2003) | [31] |
| Psychiatric symptomatology | HAM-A/HAM-D | Global screening tool; diagnostic criteria for anxiety or depression, monitoring progression and treatment response | Npt specific to PD, numerous somatic questions that overlap with PD symptoms; time consuming to administer | Hamilton (1959) Forjaz et al. (2013) Hamilton (1976) | [32] [33] [34] |
| | Parkinsons Anxiety Scale | Specific to PD, observer or patient-rated scale, diagnostic criteria for anxiety | Not as widely used, not validated for PD anxiety disease progression | Leentjens et al. (2014) | [35] |
| Fatigue | Epworth Sleepiness Scale | Global screening tool; diagnostic criteria for extreme sleepiness and monitor disease progression | Not specific to PD; does not refer to etiology of fatigue | Johns MW (1991) | [36] |
| Patient-reported outcomes | PDQ-39 | Specific to PD, widely used and patient self-administered, progression monitor for overall quality of life | Difficult to determine index score without specific calculations | Jenkinson et al. (1997) | [37] |

†List represents markers with high certainty or potentially high effect sizes developed by PDBP investigators; it does not presume to represent the entire literature. UKBB exclusion criteria for diagnosis of PD includes a family history of PD.
HAM-A/HAM-D: Hamilton Anxiety Rating Scale/Hamilton Depression Rating Scale; MDS: Movement Disorder Society; MDS-UPDRS: Movement Disorder Society-Unified Parkinson's Disease Rating Scale; MoCA: Montreal Cognitive Assessment; PDQ-39: Parkinson's Disease Questionnaire; RBD: REM behavior disorder; SCOPA-COG: Scales for Outcomes in Parkinson's Disease-Cognitive; UKBB UK Brain Bank; UPSIT: University of Pennsylvania Smell Inventory Test.

sures only motor symptoms and nonmotor symptoms are measured only by survey of the subject; the scale shows progression (worsening) of PD only very slowly such that for a typical trial of <3 years duration, insufficient worsening is seen in the placebo control patient group making it impossible to detect the existence of a modestly disease-modifying agent; the scale depends on trained raters who bring subjectivity to the assessment process; the scale is particularly susceptible to confounding by dopaminergic treatment effects. To partially address these limitations, the NIH NET-PD LS1 trial of creatine as a putative disease-modifying agent in PD used a new composite outcome measure called the global statistical test [38]. This consisted of several items in each of five clinically relevant domains (activities of daily living, cognitive function, ambulatory capacity, quality of life and global disability). Unfortunately, this study was terminated early when an interim analysis showed that continuing the trial was futile.

Convenient biomarkers may be useful in identifying asymptomatic individuals at risk for developing PD. Hyposmia is present in up to 90% of people with PD and occurs commonly years prior to the onset of motor symptoms. It also occurs in other neurodegenerative disorders and thus lacks specificity [30]. The most commonly used test to assess smell is the University of Pennsylvania Smell Identification Test [39]. This test has also been criticized from a practical standpoint for possible cultural biases, the time it takes to administer and its cost (approximately US$30/test). A recent positron emission tomography (PET) imaging study showed that odor identification is a more robust measure of forebrain cholinergic activity than odor memory/discrimination tests in PD, and odor identification tests would be the preferred olfactory method to screen for more severe cholinergic forebrain denervation in PD [40]. As it is lacking specificity, olfaction may be a more useful measure in a multimodal biomarker approach [19].

REM behavior disorder, when diagnosed by polysomnogram, is highly predictive for the development of a neurodegenerative synucleinopathy but lacks specificity for PD. In reviewing the criteria for prodromal PD, the task force assigned polysomnogram-proven REM behavior disorder a positive likelihood ratio of 130 [25]. Obtaining this measure in large populations is very difficult, however, which is why this was omitted in recent prediction models [19]. The same is true for constipation, which is present very frequently as a premotor sign in PD but lacks specificity for PD.

Impaired cognition is a major feature of advancing PD and measuring cognitive impairment may be a useful measure of disease progression or even trial end point. The Montreal Cognitive Assessment [41] was shown to be superior to other widely used clinical tools, such as the Mini-Mental State exam in that it lacks floor or ceiling effects [30]. Other neuropsychiatric tools are validated as progression measures: the Neuropsychiatric Inventory [42], Scales for Outcomes in PD-Cognition [43], the Beck Depression Scale and the Geriatric Depression Scale [44]. It is clear that clinical measures such as smell testing and sleep disorders, when added to other assessments such as genetic risk score, can be a useful component in predicting PD risk [19].

New technologies raise the prospect of collecting large amounts of 'real-world' data for assessing disease activity. Analysis of spiral drawing on a digitizing tablet offers the ability to objectively measure motor control in the ecologically relevant task of writing and discriminates early PD from controls. The use of wearable and smart-phone technology for monitoring treatment assessments has been an exciting development in PD research [45] and may prove useful for biomarker discovery. One PDBP project utilizes the APDM Mobility Lab, a sensor-based gait and balance assessment tool, to evaluate its performance as a putative state and rate biomarker platform. In a study of 135 PD subjects and 66 age-matched controls assessed at baseline, a set of gait and balance variables reported by this device correlated with PD severity measures and successfully differentiated PD subjects from controls [46]. In an ongoing study, longitudinal measures from this device are being evaluated over a period of two years in PD subjects and controls to determine if changes in parameters over time can be used as a rate biomarker for the disease.

## Imaging biomarkers

Several imaging modalities are being explored as putative biomarkers in PD. Ideal biomarkers for PD should differentiate PD from both controls as well as other forms of Parkinsonism and should reflect underlying pathological processes. It is important that any imaging biomarkers be available at most medical centers, impose minimal burden to patients and be available at a reasonable cost. Imaging markers include advances in diffusion MRI, iron MRI, functional MRI, other structural MRI techniques and developments in radiotracer imaging. (Table 5 & Supplementary Table 2)

Midbrain/nigral structural abnormalities can be evaluated using transcranial sonography, diffusion tensor magnetic resonance imaging and iron sensitive magnetic resonance imaging [41,42]. PET and SPECT ligands may be more suited to evaluation of nigrostriatal terminal dysfunction though some recent studies suggest that PET imaging of the nigra may also have advantages for assessing disease state in PD [73].

| Table 5. Most promising imaging biomarkers. | | | | |
|---|---|---|---|---|
| **Imaging** | **Total n** | **Direction** | **Study (year)** | **Ref.** |
| DatScan SPECT | 56<br>85<br>50 | Decline in [123I]β-CIT striatal uptake in PD<br>β-CIT binding reduced in PD & APS, normal in ET; declined over 1 year in PD short duration and APS, not significant in PD long duration and ET<br>decreased [123I]β-CIT over 1 year in PD | Marek *et al.* (2001)<br>Pirker *et al.* (2002)<br>Winogrodzka *et al.* (2003) | [47]<br>[48]<br>[49] |
| DMRI | (1722)<br>37<br>44<br>72<br>59<br>28 | DTI may be promising for differential diagnosis in parkinsonian symptoms<br>Lower FA in PD in neuromelanin SN ROI, not T2 ROI<br>FW values higher in PD pos SN, FW increased in PD over 1 year<br>FW values higher in ant & pos SN of PD, MSA, PSP<br>Increase in nigral MD in PD<br>FA reduced in SN in PD | Cochrane & Ebmeier (2013)<br>Langley *et al.* (2016)<br>Ofori *et al.* (2015)<br>Planetta *et al.* (2016)<br>Schwarz *et al.* (2013)<br>Vaillancourt *et al.* Neurology, (2009) | [50]<br>[51]<br>[52]<br>[53]<br>[54]<br>[55] |
| MRI | 140<br>110 | Reduced gyrification overall in PD; accelerated loss of gyrification in mid-stage PD<br>Midbrain/putaminal vol & cerebellar gray matter constructed prediction model has 97.4% diagnostic accuracy for PD vs MSA/PSP | Sterling *et al.* (2016)<br>Scherfler *et al.* (2016) | [56]<br>[57] |
| fMRI | 42<br>60<br>112<br>24<br>80<br>42<br>42 | Altered FC resting-state fMRI in distinguishing PD from controls<br>PD & PSP show hypoactivity using BOLD measures<br>PSC from task-based fMRI shows deterioration in motor cortex and putamen in PD over 1 year<br>Dopaminergic modulation of resting-state connectivity predicted dyskinesias with spec 100% and sens 91%<br>ALFF shows decreased activity in PD, deficit increases with H&Y stage<br>Increased PSC in ET, correlates w/ 3–8 Hz force oscillations<br>Reduced activation in motor control areas in MSAp and PD | Bowman *et al.* (2016)<br>Burciu *et al.* (2015)<br>Burciu *et al.* (2016)<br>Herz *et al.* (2016)<br>Luo *et al.* (2015)<br>Neely *et al.* (2015)<br>Planetta *et al.* Human Brain Mapping (2015) | [58]<br>[59]<br>[60]<br>[61]<br>[62]<br>[63]<br>[64] |
| Metabolic PET | 15<br>66<br>47<br>70 | Increased activity of PDRP over time; increased expression of PDCP over time; increased putamen metabolic activity; decreased precuneus metabolic activity<br>Decreased prefrontal and parietal metabolism and increased brainstem/cerebellar metabolism in MD-MCI; increased PDCP expression with worsening cognitive impairment<br>Correlation between PDCP and memory performance, visuospatial function and perceptual motor speed<br>Correlation between MSA, PSP and abnormal pattern expression of regional metabolic activity | Tang *et al.* (2010)<br>Huang *et al.* (2008)<br>Huang *et al.* (2007)<br>Eckert *et al.* (2008) | [65]<br>[66]<br>[67]<br>[68] |
| Cholinergic PET | 149<br>143<br>143<br>157 | Decreased thalamic cholinergic innervation in PD<br>FoG more common with diminished neocortical cholinergic innervation and increased neocortical β-amyloid deposition in PD<br>Increased caudate nucleus dopaminergic denervation and cortical cholinergic denervation in PD with more severe cognitive impairments<br>Significant slower gait speed in the low cholinergic PD subgroup | Muller *et al.* (2013)<br>Bohnen *et al.* (2014)<br>Bohnen *et al.* (2015)<br>Bohnen *et al.* (2013) | [69]<br>[70]<br>[71]<br>[72] |

Select candidate imaging markers were prioritized by the authors for inclusion in this table. This list is incomplete due to space limitations; it does not presume to represent the entire literature.
ALFF: Amplitude of low frequency fluctuation; APS: Atypical parkinsonian syndrome; BOLD: Blood oxygen level dependent; DaTScan: Dopamine transporting imaging using [123I]β-CIT; dMRI: Diffusion magnetic resonance imaging; DNH: Dorsolateral hyperintensity; ET: Essential tremor; FA: Fractional anisotropy; FC: Functional connectivity; FoG: Freezing of gait; FW: Free water; iRBD: Idiopathic rapid eye movement behavior disorder; MD: Mean diffusivity; MRI: Magnetic resonance imaging; MSA: Multiple system atrophy; PD: Parkinson's disease; PDCP: PD cognitive pattern; PDRP: PD-related pattern; PSC: Percent signal change; PSP: Progressive supranuclear palsy; SN: Substantia nigra; SPECT: Single photon emission computed tomography; SWI: Susceptibility weighted imaging.

| Imaging | Total n | Direction | Study (year) | Ref. |
|---------|---------|-----------|--------------|------|
| SWI MRI | 68 | R2* increased in SN of PD | Du *et al.* (2012) | [73] |
| | 161 | Loss of DNH in iRBD patients, similar to patients with PD | De Marzi *et al.* (2016) | [74] |
| | 210 | 88.8% sensitive and 83.6% specific for identifying parkinsonism, concordance to DatScan 86.2% | Bae *et al.* (2016) | [75] |

**Table 5. Most promising imaging biomarkers (cont.).**

Select candidate imaging markers were prioritized by the authors for inclusion in this table. This list is incomplete due to space limitations; it does not presume to represent the entire literature.
ALFF: Amplitude of low frequency fluctuation; APS: Atypical parkinsonian syndrome; BOLD: Blood oxygen level dependent; DaTScan: Dopamine transporting imaging using [123I]β-CIT; dMRI: Diffusion magnetic resonance imaging; DNH: Dorsolateral hyperintensity; ET: Essential tremor; FA: Fractional anisotropy; FC: Functional connectivity; FoG: Freezing of gait; FW: Free water; iRBD: Idiopathic rapid eye movement behavior disorder; MD: Mean diffusivity; MRI: Magnetic resonance imaging; MSA: Multiple system atrophy; PD: Parkinson's disease; PDCP: PD cognitive pattern; PDRP: PD-related pattern; PSC: Percent signal change; PSP: Progressive supranuclear palsy; SN: Substantia nigra; SPECT: Single photon emission computed tomography; SWI: Susceptibility weighted imaging.

However, detection of a dopamine deficient state may be incomplete as a biomarker in PD. Lewy bodies in PD impact not only dopamine neurons but also serotoninergic, noradrenergic and cholinergic neurons [55], and thus we need imaging biomarker(s) to capture these extranigrostriatal pathologies. Nigrostriatal terminal loss occurs also in PD mimics such as PSP, MSA and other disorders. The only FDA-approved technique for assisting in the diagnosis of PD is dopamine transporter labeling using [123I] ioflupane (DaTSCAN). Although DaTSCAN is easier to use and less expensive than many PET methods, SPECT is an intrinsically noisy imaging modality and DAT-SPECT may add little to clinical evaluation [76] PET and SPECT methods may be prone to functional modulation by symptomatic anti-PD treatment. Neither can assess nor quantify both nigrostriatal and extranigrostriatal pathological/structural changes associated with PD. This limits the ability of DaTSCAN to differentiate PD from other types of parkinsonism such as PSP [77], although it can differentiate PD from Essential Tremor [78].

One promising approach to developing an imaging biomarker would be to focus on network activity and integration using resting state fMRI (functional MRI). Changes in the default mode network have been defined in association with specific deficits [79], and further research is warranted.

### Genomic & transcriptomic biomarkers

Genomic and transcriptomic biomarkers is a category that is very broad, encompassing genetics-inspired biomarkers particularly those with potential relevance to clinical trials and practice. Much progress has been made over the last decade in elucidating the genetic architecture of PD, including more than two dozen familial genes and 24 confirmed susceptibility loci from GWAS studies [80]. These findings provide clues into the susceptibility to developing future PD in unaffected individuals. The genetic variation underlying the clinical phenotypes in patients who already have PD, however, has only very recently

begun to be addressed. *GBA* mutations, particularly those linked to severe neuropathic Gaucher's disease, have emerged as the first unequivocally and longitudinally-replicated progression variants for PD [81,82]. *GBA* mutations exert a powerful effect on cognitive decline in PD [81,82]. Targeting PD patients carrying a neuropathic *GBA* mutation should reduce sample size requirements for proof-of-concept trials focused on cognitive outcomes [81]. Moreover, α-synuclein (*SNCA*) copy number and some missense variants are associated with a fulminant clinical phenotype [82]. Conversely, some *LRRK2* mutations may correlate with milder disease phenotypes [83]. However, further longitudinal studies are needed. Other progression loci have been nominated but remain controversial and need further replication. The *APOE* e4 allele, a known risk factor for Alzheimer's disease, has been correlated with cognitive decline in PD, possibly because of co-morbid amyloidopathy in some subjects [84] but not in others [40]. The tau gene (*MAPT*) may also confer a risk for dementia in PD [85], but there is controversy [38]. To clarify the genetic architecture of disease progression in PD at genome-scale, large longitudinal or prospective efforts are needed such as that by the International Genetics of Parkinson Progression Consortium [81].

GWAS approaches are useful for identifying genetic regions that confer common risk [86,87]. Exome sequencing greatly extends the power of genetic analysis of rare variants with moderate or large effect sizes [88,89].

In addition to DNA markers, RNA markers also show promise for the diagnosis of PD. Expression of several RNA transcripts has been reported to be dysregulated in PD. SNCA transcript abundance in blood was associated with early stage and imaging-supported *de novo* PD in three independent cohorts, including HBS and PPMI [90]. Surprisingly, SNCA mRNA levels, particularly the SNCA transcripts with long 3′UTR that might target SNCA to mitochondria [91], were reduced in patients with PD. Some of the transcripts associated with PD in mul-

tiple cohorts are presented in Table 6. In addition to these transcripts, other RNAs show promise as risk, diagnostic, stratification, prognostic and progression markers, but these await further large-scale replication studies (Supplementary Table 3). RNA-sequencing studies will allow researchers to delineate the full diversity of known and novel, coding and noncoding, and long and small RNAs, detectable in circulating blood cells as well as in cell-free body fluids such as plasma and CSF.

## Proteomic & metabolomic biomarkers

This is a very broad scientific category where we consider protein markers as well as metabolomic markers, measured from diverse biofluids including plasma and CSF. As many potential markers may fit in this category, the focus of this discussion will be on markers that may be used in clinical trials or in practice in the foreseeable future, as this is the emphasis of PDBP. Because of the extensive literature in these areas, we emphasize in Table 7: markers with clear replication across cohorts; markers that

may serve as specific indicators of target engagement for therapeutics in development; and potential markers worthy of replication based on large effect sizes in early cohorts. Specific protein markers of interest include SNCA as well as others investigated based on genetic leads or unbiased screening approaches. For additional discussion of promising biomarkers, see the review by Sharma et al. [92] as well as these additional manuscripts [103–105]. Metabolic markers include metabolome profiling, as well as assays of specific enzymatic activities such as glucocerebrosidase and LRRK2 kinase activity, and specific substrate related sphingolipids for glucocerebrosidase, such as glucosylceramide and glucosylsphingosine.

Exosomes encapsulate proteins and RNAs captured from the parental cell cytosol, and thus analysis of exosomes can reveal information distinct from that of secreted proteins [123]. It is unlikely that a single protein may be an adequate marker and as such multiplexing of protein changes may be the most promising approach. Adding to the complexity of this, post-translational modifications are likely to also exert an influence.

## Table 6. Table of candidate blood transcriptional markers possibly associated with Parkinson's disease.

| Transcript | Cohorts/country | Total n[†] | Direction[‡] | Study (year) | Ref. |
|---|---|---|---|---|---|
| *SNCA* (including long 3'UTR-SNCA) | HBS | 405 | Reduced in early-stage and *de novo* PD | Locascio et al. (2015) | [92] |
| | PPMI | 340 | | Locascio et al. (2015) | [92] |
| | PROBE | 120 | Reduced in *de novo* PD | Locascio et al. (2015) | [92] |
| | Portugal | 67 | Reduced in PD | Pinho et al. (2016) | [93] |
| | Sweden[§] | 154 | Reduced in fast progressing PD vs slow progressing PD | Karlsson et al. (2013) | [94] |
| | USA | 105[¶] | Associated with PD Reduced in PD[¶] | Shehadeh et al. (2010) | [95] |
| *COPZ1* | PROBE | 124 | Increased in PD | Potashkin et al. (2012) | [96] |
| | HBS | 96 | Increased in PD | Santiago et al. (2013) | [97] |
| | PPMI | 200 | Increased in *de novo* PD | Santiago & Potaskin, (2015) | [98] |
| *ALDH1A1* | Germany | 153 | Associated with PD[§] | Grunblatt et al. (2010) | [99] |
| | EU | 185 | Reduced in PD | Molochnikov et al. (2012) | [100] |
| | Italy | 24 | Reduced in *de novo* PD | Calligaris et al. (2015) | [101] |
| *LRPPRC* | USA | 48 | Reduced in early and *de novo* PD | Scherzer et al. (2007) | [102] |
| | Sweden | 154 | Associated with PD[§] | Karlsson et al. (2013) | [94] |
| *BCL2* | USA | 48 | Reduced in early and *de novo* PD | Scherzer et al. (2007) | [102] |
| | USA | 28 | Reduced in PD | Shehadeh et al. (2010) | [95] |
| | Sweden | 154 | Associated with PD[§] | Karlsson et al. (2013) | [94] |
| *BCL11B* | USA | 28 | Reduced in early and *de novo* PD | Scherzer et al. (2007) | [102] |
| | USA | 48 | Reduced in PD | Shehadeh et al. (2010) | [95] |
| *APP* | PROBE | 95 | Increased in PD | Santiago et al. (2013) | [97] |
| | HBS | 96 | Increased in PD | Santiago et al. (2013) | [97] |

[†]Total n includes number of patients with PD and controls assayed.
[‡]Direction for transcripts significantly differentially expressed; study-specific significance criteria were used.
[§]Direction of change not mentioned.
[¶]Select candidate transcripts, who met study-specific significance criteria with same directional change in at least two cohorts, were prioritized by the authors for inclusion in this table. This list is incomplete due to space limitations; it does not presume to represent the entire literature.
This is a reanalysis of the Scherzer, *PNAS* (2007) [102] microarray dataset performed by Shehadeh, *PLoS ONE* (2010) [95].
D: Parkinson's disease.

| Table 7. Table of candidate protein biomarkers for Parkinson's disease. | | | | | | |
|---|---|---|---|---|---|---|
| Marker | Biofluid | >100/ group | Replicated | Synopsis of result | Study (year) | Ref. |
| EGF | Plasma/ serum | Y | Y | Lower EGF predicts cognitive decline, but effect is modest | Chen-Plotkin *et al.* (2011) Pellecchia *et al.* (2013) Lim *et al.* (2016) | [106] [107] [108] |
| Aβ, PTau, TTau | CSF | Y | Y | Lower Aβ and higher P-tau may predict cognitive decline | Siderowf *et al.* (2010) Zhang *et al.* (2013) | [109] [110] |
| Total ASyn | CSF | Y | Y | Lower α-synuclein predicts better preservation of cognitive function | Stewart *et al.* (2014) | [111] |
| ApoA1 | Plasma/ serum | Y | Y | Lower ApoA1 levels correlate with earlier age at onset and greater disease severity | Qiang *et al.* (2013) Swanson *et al.* (2015) | [112] [113] |
| Vit D | Plasma/ serum | Y | Y | More Vit D insufficiency in PD vs controls Lower Vit D correlates with increased risk of developing PD | Evatt *et al.* (2008) Knekt *et al.* (2010) Ding *et al.* (2013) | [114] [115] [116] |
| Urate | Plasma/ serum | Y | Y | Higher urate possibly protective, especially in men. In trials | Schwarzschild *et al.* (2008) Ascherio *et al.* (2009) | [117] [118] |
| Panel of 21 proteins | Serum | Y | N | 21-protein panel differentiates PD vs AD vs control. High accuracy | O'Bryant *et al.* (2014) | [119] |
| Panel of 7 proteins (ASyn, DJ1, PTau, TTau, Aβ, Flt3Ligand, fractalkine) | CSF | N | N | PD vs control sens 92%/spec 60% PD vs MSA sens 99%/spec 90% PD vs AD sens 92%/spec 84% | Shi *et al.* (2011) | [120] |
| Panel of 6 proteins/ peptides (SPP1, LRP1, CSF1R, EPH4, TIMP1, APLP1) | CSF | N | N | AUC 0.85 differentiating PD vs controls | Shi *et al.* (2015) | [121] |
| AD-derived markers (CSF Aβ, PTau, TTau, MRI, *APOE* genotype) | Multimodal | N | N | AUC 0.87 differentiating PD with normal cognition vs PD with dementia | Berlyand *et al.* (2016) | [122] |

Shaded lines indicate candidates tested in larger samples with replication (results are more certain). Other markers, for which results are less certain, are included if the reported effect size is large. List represents markers with high certainty or potentially high effect sizes developed by PDBP investigators; it does not presume to represent the entire literature.
CSF: Cerebrospinal fluid.

## Recommendations for the future

The PDBP Consortium has proposed several recommendations for the field of PD Biomarkers based on the review of the activities of the field as summarized above.

## Harmonization

We recommend continued harmonization of clinical and laboratory data and biospecimen collection. The PDBP DMR has been created and provides essential infrastructure to the project to allow harmonization to continue. The clinical measures are standardized via the use of shared assessments and clinical data elements across studies in the DMR. Additional harmonization efforts have been essential in PDBP in terms of standardized operating procedures for the collection and storage of biospecimens. The standard operating procedures can be found online [124]. The BioSEND Repository houses not only PDBP and BioFIND samples, but also the PPMI samples, which will facilitate across all PD-based biomarker initiatives standardized collection, handling and request for access practices as noted above [22]. Additional challenges with harmonization include the identification of the same biomarker

with different technologies or methodologies. Again, standardized operating procedures can assist with this challenge as can the process of identifying the best and most widely reproducible methodology.

## Replication

We also recommend continued replication. Replication of results, as noted above, is essential for moving biomarkers projects forward toward clinical trial and practice usefulness. Toward this goal, the PDBP has released a Funding Opportunity Announcement (FOA) in the past to allow for replication of discoveries using the PDBP samples and data collection; future opportunities are anticipated [125]. To date, seven replication projects have been funded under PDBP mechanisms, and others additionally have been approved via the PD BRAC. One of these projects has led to a publication in which PDBP has been used as a replication sample [19].

## Publication of negative results

We recommend a systematic method for reporting and collating negative results. The phenomenon of bias toward positive results in the literature has been noted [126] and it arguably presents a particularly significant obstacle toward biomarkers replication/validation. Negative results, especially from replication/validation studies are essential to inform the biomarkers process. Some journals publish and encourage negative results (such as *PLoS ONE* [127] and *eNeuro* [128]), and we urge the use of these forums for dissemination of negative and of failure to replicate studies in these and other journals. Ideally, both positive and negative studies could be collated and managed in a searchable resource. A data management solution, which does not yet exist but can be envisioned, where findings could be surveyed, would be an extremely useful tool to biomarkers researchers.

## Use of *de novo* cohorts for discovery efforts & longitudinal follow-up of well-characterized cohorts

There are two important cohorts that are necessary to identify biomarkers. First, creation of a cohort of newly diagnosed individuals for discovery of biomarkers would add to the current biomarker landscape. Second, we should continue to follow-up existing cohorts to determine their clinical outcomes.

The value of large, pragmatic trials is increasingly being recognized [2]. The NINDS PDBP was designed to reflect the real-life clinical situation in its recruitment, inclusion/exclusion criteria and assessment goals [10]. As a result, PDBP subjects are typically on symptomatic treatment. However, the importance of the use of *de novo*, or newly diagnosed, cohorts for discovery remains an unmet need. There are challenges to collecting and studying *de novo* PD patients that are both ethical as well as sample-size related. PPMI has successfully recruited and followed over 400 *de novo* subjects with clinical PD. However, to our knowledge, these samples and data are approved for validation and qualification use. Expanding the scope of this and other cohorts, such as the DeNoPa cohort [44] and other international PD cohorts [129] to support discovery biomarker research would be a boon to the field.

The original PDBP FOA was designed for three or more years of follow-up for each study. Considering the long clinical course and relatively slow rate of decline in PD, longitudinal follow-up will inform hypotheses tested and data collected on the PDBP cohort. Challenges in accomplishing this include the cost for long-term follow-up, especially for individuals where no intervention nor hypothesis testing is occurring. Strategies for overcoming this obstacle include novel approaches to collecting meaningful longitudinal data in a cost-effective manner such as the use of phone survey(s), wearable technology approaches and virtual care visits.

There are validated phone surveys for use in PD [130]. These could be utilized for periodic follow-up, alone or in combination with other tools. Wearables are particularly adaptable to the measure of tremor and movement and have the advantage of convenience and likely good compliance [131]. Telemedicine is used in clinical care, as well as in some research endeavors [132]. It could be possible to extend the use of telemedicine into a well-defined longitudinal follow-up collection of data on the PDBP and other cohorts toward the goal of enriching the outcome information of existing biospecimen collections.

The need for expanding the types of biospecimens was also discussed. As postmortem evaluation remains the gold standard in PD and other neurodegenerative diseases, and because several sites in the PDBP have an autopsy program (via the NINDS-funded Udall Centers of Excellence as well as at other clinical sites), the committee recommended dovetailing brain banking with the ongoing PDBP activities. For instance, the Neurobiobank, which is supported by the National Institute of Mental Health, NINDS and the Eunice Kennedy Shriver National Institute of Child Health and Human Development, brings together multiple stakeholders to facilitate research advancement through the collection and distribution of human postmortem brain tissue [133]. While this resource is currently available to PDBP researchers, a formalized integration could also be pursued. Common standards for brain collection, preparation and neuropathologic analysis should be developed, per-

haps along the lines used by the National Alzheimer's Coordinating Center.

Additional biologicals to consider for banking include resources for the development of induced pluripotent stem cells. Although the potential of this cell resource for future drug discovery efforts is significant, the banking of fibroblasts and peripheral blood mononuclear cells should be prioritized in order to assure leveraging of resources. Some possible subtypes within the PDBP and other cohorts could include: those with extremes in clinical measures, patients with a change in clinical diagnosis, those who meet the Nalls criteria for risk [19] and those with genetic components as risk factors or causal mutations.

Additional future directions include biomarkers studies on cognitive impairment in Parkinsonism. Cognitive impairment, including dementia, is commonly seen in those with PD. Pathologically, PD with dementia (PDD) is most often associated with the presence of cortical Lewy bodies, as is the closely related dementia with Lewy bodies [134]. Clinically, however, the two disorders are not always distinct. Biologically, there is clear overlap and some controversy regarding biological differences between PDD and Dementia with Lewy Bodies (DLB). The PDBP is funding four new studies under a recently released FOA directed at furthering discovery science in PDD and DLB biomarkers, including building additional clinical cohorts with well-characterized subjects and standardized clinical specimens banked via BioSEND.

Early in the disease course, it can be difficult to differentiate PD from other conditions such as PSP and MSA. Even with the correct diagnosis, PD has wide variability in disease course, rate of progression and response to treatment, making an objective marker extremely useful [135]. Therefore, in response to this need, NINDS has also recently released an FOA (PAR 16–112) toward discovery of biomarkers differentiating PD from others forms of Parkinsonism and from ET. This FOA also seeks to recruit subjects in discovery projects toward a better understanding of ethnic, genetic and other subtypes and stratifications within the PD patient population.

Incorporation of larger datasets, such as those associated with whole genome sequencing will require a movement from a server-based data repository to a cloud-based solution. Storage and access of data in the cloud will also facilitate a new approach to data handling where funding support is needed for cloud-based assessments and collaborative types of studies. Data types generated through biomarker discovery platforms require the development of standard analysis pipelines to enable data to be readily shared across studies. The inclusion of information on the pipeline used (providence) in the data management resource will facilitate the replication of biomarker results.

Determining PD biomarkers from diverse data sources depends critically on the use of rigorous analytic techniques. First, it is imperative for methods to yield not only accurate biomarkers but also ones that are highly reliable, that is, likely to attain comparable accuracy in other samples and across discovery, replication/validation and qualification phases. To achieve such reliability, reproducible methods should be used, ranging from data processing to software to the choice of statistical methods. Second, several of the data modalities in the PDBP DMR involve large-scale high throughput measures such as GWAS, proteomic, metabolomics and various neuroimaging techniques. Statistical methods applied should be able to cope with high-dimensional data, addressing issues such as multiple testing adjustments for error control, multicollinearity, overfitting and variable selection. Third, the PDBP DMR and similar resources provide rare opportunities for access to extremely rich data, potentially enabling the combination of different data types, which may reveal distinct manifestations of PD pathology. The data should be fully leveraged, when appropriate, to integrate across different data types and to conduct multimodal analyses. Suitable tools to investigate PD biomarkers using large-scale multimodal data are beginning to emerge [136,137], but there is an important need to ensure that proper methods are applied and, in some cases, to develop new statistical techniques.

## Conclusion

The PDBP has accomplished its originally stated goals of: creating data management infrastructure, evaluating clinical cohorts with standardized biospecimen collection and establishing laboratory-based and clinical discovery science [7]. Through this process, it has become clear that the 'basic research' part of the pipeline is actually quite complex. Better description and evaluation of this part of the process may ultimately lead to more qualification of biomarkers generally and for PD in particular. Negative results in both discovery and replication are an essential component of the biomarkers process, at every stage, but perhaps especially throughout the discovery (Basic Research) phase. There are no defined US FDA Biomarkers entry criteria. So, while keeping the end of FDA qualification in mind is important, it is a difficult target. Consideration of final use and application of PD biomarkers is crucial when planning biomarker discovery projects as well as data management and biospecimen collection. Key to this planning activity is standardization of clinical data collection, and of biospecimen collection and handling.

It is unlikely that a single marker will provide sufficient sensitivity and specificity for early diagnosis or

prognosis. A model developed by Nalls *et al.* [19] showed that olfactory function, genetic risk, family history of PD, age and gender were able to differentiate cases from controls in the PPMI sample, and this finding was replicated in the PDBP and other sample sets [19]. We believe that a combination of different biomarkers will be vital for the development of objective end points for future neuroprotection trials. In order to create this multimodal approach to biomarkers, new analysis and data integration methods and approaches will be needed. Sleep and imaging measures, and to some extent nonmotor symptoms, assessed using adequate scales, may be more informative markers to quantify progression [44]. The PDBP will continue to study these markers and seek to identify markers for PDD and other causes of parkinsonism and dementia while also supporting the fundamental need for replication of discoveries in PD biomarker research.

---

## Executive summary

**Potential role of biomarkers**
- Biomarkers are needed to improve the diagnosis and treatment of Parkinson's disease (PD).
- To be approved for use in clinical practice in the United States, biomarkers must be replicated, validated and then qualified through the US FDA.

**Parkinson's Disease Biomarker Program's achievements**
- The National Institute of Neurological Disorders and Stroke Parkinson's Disease Biomarker Program (PDBP) has established a central data repository for all PD biomarker research and a central biorepository for blood and cerebrospinal fluid samples.

**Overview of promising biomarkers**
- While there are promising clinical, imaging, metabolomic, proteomic and genomic markers identified by the PDBP and other cohorts, no PD biomarker has been qualified by the FDA.

**Recommendations for the future**
- Biomarker identification could be improved through continued harmonization of clinical assessments and biosample ascertainment. Use of the PDBP Data Management Resource and the PDBP standard operating procedures can facilitate this goal.
- Research laboratories and medical journal publishers should place greater emphasis on the replication of promising results and publication of negative findings.
- Use of cohorts of individuals that are newly diagnosed as well as continued longitudinal follow-up of existing well-characterized cohorts are integral to biomarker development.

## References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1    Seidl SE, Potashkin JA. The promise of neuroprotective agents in Parkinson's disease. *Front. Neurol.* 2, 68 (2011).

2    Meissner WG, Frasier M, Gasser T *et al.* Priorities in Parkinson's disease research. *Nat. Rev. Drug Discov.* 10(5), 377–393 (2011).

3    Macleod AD, Taylor KSM, Counsell CE. Mortality in Parkinson's disease: a systematic review and meta-analysis. *Mov. Disord.* 29(13), 1615–1622 (2014).

4    Kotagal V, Albin RL, Müller MLTM, Koeppe RA, Frey KA, Bohnen NI. Modifiable cardiovascular risk factors and axial motor impairments in Parkinson disease. *Neurology* 82(17), 1514–1520 (2014).

5    Williams-Gray CH, Mason SL, Evans JR *et al.* The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *J. Neurol. Neurosurg. Psychiatry* 84(11), 1258–1264 (2013).

6    PD Biomarkers Strategic Planning Workshop.  www.ninds.nih.gov/funding/areas/neurodegeneration

7    NINDS Parkinson's Disease Biomarkers Program. Scientific liaison group meeting. www.ninds.nih.gov/research/parkinsonsweb/PD

8    Marek K, Jennings D, Lasch S *et al.* The Parkinson progression marker initiative (PPMI). *Prog. Neurobiol.* 95(4), 629–635 (2011).

9    Kang UJ, Goldman JG, Alcalay RN *et al.* The BioFIND study: characteristics of a clinically typical Parkinson's disease biomarker cohort. *Mov. Disord.* 31(6), 924–932 (2016).

10   Rosenthal LS, Drake D, Alcalay RN *et al.* The NINDS Parkinson's disease biomarkers program. *Mov. Disord.* 31(6), 915–923 (2016).

11   FDA-NIH Biomarker Working Group. *BEST (Biomarkers, EndpointS, and Other Tools) Resource.* US FDA, MD, USA, and NIH, MD, USA (2016).

12   McCune S. *Outcome Measures, Biomarkers and Endpoints.* US FDA, MD, USA. http://www.fda.gov/downloads/NewsEvents/Meetings

13   Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat. Biotechnol.* 24(8), 971–983 (2006).

•    **Describes the advances and needs in the field of proteomics and how to go about identifying Parkinson's disease (PD) biomarkers.**

14   Drug Development Tools Qualification Programs – Biomarker Qualification Program. US FDA, MD, USA www.fda.gov/Drugs/DevelopmentApprovalProcess/

15   Grinnon ST, Miller K, Marler JR *et al.* National Institute of Neurological Disorders and Stroke Common Data Element Project – approach and methods. *Clin. Trials* 9(3), 322–329 (2012).

16   He L, Lee E-Y, Sterling NW *et al.* The key determinants to quality of life in Parkinson's disease patients: results from the Parkinson's Disease Biomarker Program (PDBP). *J. Parkinsons. Dis.* 6(3), 523–532 (2016).

17   Mills KA, Mari Z, Pontone GM *et al.* Cognitive impairment in Parkinson's disease: association between patient-reported and clinically measured outcomes. *Parkinsonism Relat. Disord.* 33, 107–114 (2016).

18   Ofori E, Du G, Babcock D, Huang X, Vaillancourt DE. Parkinson's disease biomarkers program brain imaging repository. *Neuroimage* 124(Pt B), 1120–1124 (2016).

•    **Describes the Parkinson's Disease Biomarker Program (PDBP) Data Management Resource, a publicly available database of the clinical assessments and imaging of all patients in the PDBP.**

19   Nalls MA, McLean CY, Rick J *et al.* Diagnosis of Parkinson's disease on the basis of clinical and genetic classification: a population-based modelling study. *Lancet Neurol.* 14(10), 1002–1009 (2015).

•    **Used large patient cohorts (PPMI, PDBP, Parkinson's Associated Risk Study, 23andme, Longitudinal and Biomarker Study in PD and the Udall Center at the University of Pennsylvania) to develop and test a statistical model that differentiates individuals with PD from control.**

20   Mueller SG, Weiner MW, Thal LJ *et al.* Ways toward an early diagnosis in Alzheimer's disease: the Alzheimer's Disease Neuroimaging Initiative (ADNI). *Alzheimers. Dement.* 1(1), 55–66 (2005/7).

21   Program PDB. Application for access to Parkinson's disease biomarker samples application for access to Parkinson's disease biomarker samples (2016). https://pdbp.ninds.nih.gov/content/application-webform

22   BioSEND. www.biosend.org/

23   Nalls MA, Bras J, Hernandez DG *et al.* NeuroX, a fast and efficient genotyping platform for investigation of neurodegenerative diseases. *Neurobiol. Aging.* 36(3), 1605. e7–1605.e12 (2015).

24   Xiao Q, Chen S, Le W. Hyposmia: a possible biomarker of Parkinson's disease. *Neurosci. Bull.* 30(1), 134–140 (2014).

25   Berg D, Postuma RB, Adler CH *et al.* MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* 30(12), 1600–1611 (2015).

26   Iranzo A, Molinuevo JL, Santamaría J *et al.* Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet Neurol.* 5(7), 572–577 (2006).

27  Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. *Neurology* 42(6), 1142–1146 (1992).

28  Postuma RB, Berg D, Stern M *et al.* MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* 30(12), 1591–1601 (2015).

29  Goetz CG, Tilley BC, Shaftman SR *et al.* Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* 23(15), 2129–2170 (2008).

30  Dalrymple-Alford JC, MacAskill MR, Nakas CT *et al.* The MoCA: well-suited screen for cognitive impairment in Parkinson disease. *Neurology* 75(19), 1717–1725 (2010).

31  Marinus J, Visser M, Verwey NA *et al.* Assessment of cognition in Parkinson's disease. *Neurology* 61(9), 1222–1228 (2003).

32  Hamilton M. The assessment of anxiety states by rating. *Br. J. Med. Psychol.* 32(1), 50–55 (1959).

33  Forjaz MJ, Martinez-Martin P, Dujardin K *et al.* Rasch analysis of anxiety scales in Parkinson's disease. *J. Psychosom. Res.* 74(5), 414–419 (2013).

34  Hamilton M. Comparative value of rating scales. *Br. J. Clin. Pharmacol.* 3(1 Suppl. 1), 58–60 (1976).

35  Leentjens AFG, Dujardin K, Pontone GM, Starkstein SE, Weintraub D, Martinez-Martin P. The Parkinson Anxiety Scale (PAS): development and validation of a new anxiety scale. *Mov. Disord.* 29(8), 1035–1043 (2014).

36  Johns MW, Sleep Disorders Unit. A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. *Sleep* 14(6), 540–545 (1991).

37  Jenkinson C, Fitzpatrick R, Peto V, Greenhall R, Hyman N. The Parkinson's Disease Questionnaire (PDQ-39): development and validation of a Parkinson's disease summary index score. *Age Ageing* 26(5), 353–357 (1997).

38  Elm JJ, NINDS NET-PD Investigators. Design innovations and baseline findings in a long-term Parkinson's trial: the National Institute of Neurological Disorders and Stroke Exploratory Trials in Parkinson's Disease Long-Term Study-1. *Mov. Disord.* 27(12), 1513–1521 (2012).

39  Doty RL, Paul S, Michael D. Development of the university of pennsylvania smell identification test: a standardized microencapsulated test of olfactory function. *Physiol. Behav.* 32(3), 489–502 (1984).

40  Bohnen N, Albin R, Koeppe R, Frey K, Muller M. Odor identification is a more robust measure of forebrain cholinergic activity than odor memory/discrimination tests in Parkinson disease. *J. Nucl. Med.* 54(Suppl. 2), 1786–1786 (2013).

41  Nasreddine ZS, Phillips NA, Bédirian V *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53(4), 695–699 (2005).

42  Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44(12), 2308–2314 (1994).

43  Isella V. Psychometric properties of the Italian version of the Scales for Outcomes in Parkinson's Disease-in Parkinson's Disease-Cognition (SCOPA-Cog). *Funct. Neurol.* 28(2), 121–125 (2013).

44  Mollenhauer B, Zimmermann J, Sixel-Döring F *et al.* Monitoring of 30 marker candidates in early Parkinson disease as progression markers. *Neurology* 87(2), 168–177 (2016).

45  Pastorino M, Arredondo MT, Cancela J, Guillen S. Wearable sensor network for health monitoring: the case of Parkinson disease. *J. Phys. Conf. Ser.* 450, 012055 (2013).

46  Dewey DC, Miocinovic S, Bernstein I *et al.* Automated gait and balance parameters diagnose and correlate with severity in Parkinson disease. *J. Neurol. Sci.* 345(1–2), 131–138 (2014).

47  Marek K, Innis R, van Dyck C *et al.* [123I]beta-CIT SPECT imaging assessment of the rate of Parkinson's disease progression. *Neurology* 57(11), 2089–2094 (2001).

48  Pirker W, Djamshidian S, Asenbaum S *et al.* Progression of dopaminergic degeneration in Parkinson's disease and atypical parkinsonism: a longitudinal beta-CIT SPECT study. *Mov. Disord.* 17(1), 45–53 (2002).

49  Winogrodzka A, Bergmans P, Booij J, van Royen EA, Stoof JC, Wolters EC. [(123)I]beta-CIT SPECT is a useful method for monitoring dopaminergic degeneration in early stage Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 74(3), 294–298 (2003).

50  Cochrane CJ, Ebmeier KP. Diffusion tensor imaging in parkinsonian syndromes: a systematic review and meta-analysis. *Neurology* 80(9), 857–864 (2013).

51  Langley J, Huddleston DE, Merritt M *et al.* Diffusion tensor imaging of the substantia nigra in Parkinson's disease revisited. *Hum. Brain Mapp.* 37(7), 2547–2556 (2016).

52  Ofori E, Pasternak O, Planetta PJ *et al.* Longitudinal changes in free-water within the substantia nigra of Parkinson's disease. *Brain* 138(Pt 8), 2322–2331 (2015).

53  Planetta PJ, Ofori E, Pasternak O *et al.* Free-water imaging in Parkinson's disease and atypical parkinsonism. *Brain* 139(Pt 2), 495–508 (2016).

54  Schwarz ST, Abaei M, Gontu V, Morgan PS, Bajaj N, Auer DP. Diffusion tensor imaging of nigral degeneration in Parkinson's disease: a region-of-interest and voxel-based study at 3 T and systematic review with meta-analysis. *NeuroImage Clinical* 3, 481–488 (2013).

55  Brooks DJ, Pavese N. Imaging biomarkers in Parkinson's disease. *Prog. Neurobiol.* 95(4), 614–628 (2011).

56  Sterling NW, Wang M, Zhang L *et al.* Stage-dependent loss of cortical gyrification as Parkinson disease "unfolds." *Neurology* 86(12), 1143–1151 (2016).

57  Scherfler C, Göbel G, Müller C *et al.* Diagnostic potential of automated subcortical volume segmentation in atypical parkinsonism. *Neurology* 86(13), 1242–1249 (2016).

58  Bowman FD, Drake DF, Huddleston DE. Multimodal imaging signatures of Parkinson's disease. *Front. Neurosci.* 10, 131 (2016).

59  Burciu RG, Ofori E, Shukla P *et al.* Distinct patterns of brain activity in progressive supranuclear palsy and Parkinson's disease. *Mov. Disord.* 30(9), 1248–1258 (2015).

60    Burciu RG, Chung JW, Shukla P *et al.* Functional MRI of disease progression in Parkinson disease and atypical parkinsonian syndromes. *Neurology* 87(7), 709–717 (2016).

61    Herz DM, Haagensen BN, Nielsen SH, Madsen KH, Løkkegaard A, Siebner HR. Resting-state connectivity predicts levodopa-induced dyskinesias in Parkinson's disease. *Mov. Disord.* 31(4), 521–529 (2016).

62    Luo C, Guo X, Song W *et al.* The trajectory of disturbed resting-state cerebral function in Parkinson's disease at different Hoehn and Yahr stages. *Hum. Brain Mapp.* 36(8), 3104–3116 (2015).

63    Neely KA, Kurani AS, Shukla P *et al.* Functional brain activity relates to 0–3 and 3–8 Hz force oscillations in essential tremor. *Cereb. Cortex* 25(11), 4191–4202 (2015).

64    Planetta PJ, Kurani AS, Shukla P *et al.* Distinct functional and macrostructural brain changes in Parkinson's disease and multiple system atrophy. *Hum. Brain Mapp.* 36(3), 1165–1179 (2015).

65    Tang CC, Poston KL, Dhawan V, Eidelberg D. Abnormalities in metabolic network activity precede the onset of motor symptoms in Parkinson's disease. *J. Neurosci.* 30(3), 1049–1056 (2010).

66    Huang C, Mattis P, Perrine K, Brown N, Dhawan V, Eidelberg D. Metabolic abnormalities associated with mild cognitive impairment in Parkinson disease. *Neurology* 70(16 Pt 2), 1470–1477 (2008).

67    Huang C, Mattis P, Tang C, Perrine K, Carbon M, Eidelberg D. Metabolic brain networks associated with cognitive function in Parkinson's disease. *Neuroimage* 34(2), 714–723 (2007).

68    Eckert T, Tang C, Ma Y *et al.* Abnormal metabolic networks in atypical parkinsonism. *Mov. Disord.* 23(5), 727–733 (2008).

69    Müller MLTM, Albin RL, Kotagal V *et al.* Thalamic cholinergic innervation and postural sensory integration function in Parkinson's disease. *Brain* 136(Pt 11), 3282–3289 (2013).

70    Bohnen NI, Frey KA, Studenski S *et al.* Extra-nigral pathological conditions are common in Parkinson's disease with freezing of gait: an *in vivo* positron emission tomography study. *Mov. Disord.* 29(9), 1118–1124 (2014).

71    Bohnen NI, Albin RL, Müller MLTM *et al.* Frequency of cholinergic and caudate nucleus dopaminergic deficits across the predemented cognitive spectrum of Parkinson disease and evidence of interaction effects. *JAMA Neurol.* 72(2), 194–200 (2015).

72    Bohnen NI, Frey KA, Studenski S *et al.* Gait speed in Parkinson disease correlates with cholinergic degeneration. *Neurology* 81(18), 1611–1616 (2013).

73    Hansen AK, Knudsen K, Lillethorup TP *et al. In vivo* imaging of neuromelanin in Parkinson's disease using 18F-AV-1451 PET. *Brain.* 139(Pt 7), 2039–2049 (2016).

74    De Marzi R, Seppi K, Högl B *et al.* Loss of dorsolateral nigral hyperintensity on 3.0 tesla susceptibility-weighted imaging in idiopathic rapid eye movement sleep behavior disorder. *Ann. Neurol.* 79(6), 1026–1030 (2016).

75    Bae YJ, Kim J-M, Kim E *et al.* Loss of nigral hyperintensity on 3 Tesla MRI of parkinsonism: comparison with (123) I-FP-CIT SPECT. *Mov. Disord.* 31(5), 684–692 (2016).

76    Xie T, Warnke P, Kang UJ, de la Fuente-Fernandez R. Role of DaTSCAN and clinical diagnosis in Parkinson disease. *Neurology.* 79(16), 1744; Author reply 1744 (2012).

77    Kim YJ, Ichise M, Ballinger JR *et al.* Combination of dopamine transporter and D2 receptor SPECT in the diagnostic evaluation of PD, MSA, and PSP. *Mov. Disord.* 17(2), 303–312 (2002).

78    Marshall VL, Reininger CB, Marquardt M *et al.* Parkinson's disease is overdiagnosed clinically at baseline in diagnostically uncertain cases: a 3-year European multicenter study with repeat [123I]FP-CIT SPECT. *Mov. Disord.* 24(4), 500–508 (2009).

79    Lucas-Jiménez O, Ojeda N, Peña J *et al.* Altered functional connectivity in the default mode network is associated with cognitive impairment and brain anatomical changes in Parkinson's disease. *Parkinsonism Relat. Disord.* 33, 58–64 (2016).

80    Trinh J, Farrer M. Advances in the genetics of Parkinson disease. *Nat. Rev. Neurol.* 9(8), 445–454 (2013).

81    Liu G, Boot B, Locascio JJ *et al.* Specifically neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Ann. Neurol.* 80(5), 674–685 (2016).

82    Cilia R, Roberto C, Sara T *et al.* Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann. Neurol.* 80(5), 662–673 (2016).

83    Healy DG, Falchi M, O'Sullivan SS *et al.* Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.* 7(7), 583–590 (2008).

84    Pankratz N, Byder L, Halter C *et al.* Presence of an APOE4 allele results in significantly earlier onset of Parkinson's disease and a higher risk with dementia. *Mov. Disord.* 21(1), 45–49 (2006).

85    Mata IF, Leverenz JB, Weintraub D *et al.* APOE, MAPT, and SNCA genes and cognitive performance in Parkinson disease. *JAMA Neurol.* 71(11), 1405–1412 (2014).

86    Simón-Sánchez J, Schulte C, Bras JM *et al.* Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* 41(12), 1308–1312 (2009).

87    Nalls MA, Pankratz N, Lill CM *et al.* Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat. Genet.* 46(9), 989–993 (2014).

88    Jansen IE, Ye H, Heetveld S *et al.* Discovery and functional prioritization of Parkinson's disease candidate genes from large-scale whole exome sequencing. *Genome Biol.* 18(1), 22 (2017).

89    Guerreiro R, Brás J, Hardy J, Singleton A. Next generation sequencing techniques in neurological diseases: redefining clinical and molecular associations. *Hum. Mol. Genet.* 23(R1), R47–R53 (2014).

90    Locascio JJ, Eberly S, Liao Z *et al.* Association between α-synuclein blood transcripts and early, neuroimaging-supported Parkinson's disease. *Brain* 138(Pt 9), 2659–2671 (2015).

91    Rhinn H, Qiang L, Yamashita T *et al.* Alternative α-synuclein transcript usage as a convergent mechanism in Parkinson's disease pathology. *Nat. Commun.* 3, 1084 (2012).

•    **Describes specific variants of α-synuclein that are more pathologic than other variants and the subsequent novel mechanism of alpha-synuclein regulation that occurs in PD.**

92    Sharma S, Moon CS, Khogali A *et al.* Biomarkers in Parkinson's disease (recent update). *Neurochem. Int.* 63(3), 201–229 (2013).

93    Pinho R, Guedes LC, Soreq L *et al.* Gene expression differences in peripheral blood of Parkinson's disease patients with distinct progression profiles. *PLoS ONE* 11(6), e0157852 (2016).

94    Karlsson MK, Sharma P, Aasly J *et al.* Found in transcription: accurate Parkinson's disease classification in peripheral blood. *J. Parkinsons. Dis.* 3(1), 19–29 (2013).

95    Shehadeh LA, Yu K, Wang L *et al.* SRRM2, a potential blood biomarker revealing high alternative splicing in Parkinson's disease. *PLoS ONE* 5(2), e9104 (2010).

96    Potashkin JA, Santiago JA, Ravina BM, Watts A, Leontovich AA. Biosignatures for Parkinson's disease and atypical parkinsonian disorders patients. *PLoS ONE* 7(8), e43595 (2012).

97    Santiago JA, Potashkin JA. Integrative network analysis unveils convergent molecular pathways in Parkinson's disease and diabetes. *PLoS ONE* 8(12), e83940 (2013).

98    Santiago JA, Potashkin JA. Blood biomarkers associated with cognitive decline in early stage and drug-naive Parkinson's disease patients. *PLoS ONE* 10(11), e0142582 (2015).

99    Grünblatt E, Zehetmayer S, Jacob CP, Müller T, Jost WH, Riederer P. Pilot study: peripheral biomarkers for diagnosing sporadic Parkinson's disease. *J. Neural Transm.* 117(12), 1387–1393 (2010).

100    Molochnikov L, Rabey JM, Dobronevsky E *et al.* A molecular signature in blood identifies early Parkinson's disease. *Mol. Neurodegener.* 7(1), 26 (2012).

101    Calligaris R, Banica M, Roncaglia P *et al.* Blood transcriptomics of drug-naïve sporadic Parkinson's disease patients. *BMC Genomics* 16, 876 (2015).

102    Scherzer CR, Eklund AC, Morse LJ *et al.* Molecular markers of early Parkinson's disease based on gene expression in blood. *Proc. Natl Acad. Sci. USA* 104(3), 955–960 (2007).

103    Sharma SK, Carlson EC, Ebadi M. Neuroprotective actions of selegiline in inhibiting 1-methyl, 4-phenyl, pyridinium ion (MPP+)-induced apoptosis in SK-N-SH neurons. *J. Neurocytol.* 32(4), 329–343 (2003).

104    Sharma SK. *Progress in PET Radiopharmaceuticals: Quality Control and Theranostics.* Nova Science Publishers, Inc. NY, USA (2016).

105    Sharma S, editor. Biomarkers in personalized theranostics of Parkinson's disease. In: *Personalized Medicine (Beyond Pet Biomarkers).* Nova Science Publishers, NY, USA, 217–236 (2016).

106    Chen-Plotkin AS, Hu WT, Siderowf A *et al.* Plasma epidermal growth factor levels predict cognitive decline in Parkinson disease. *Ann. Neurol.* 69(4), 655–663 (2011).

107    Pellecchia MT, Santangelo G, Picillo M *et al.* Serum epidermal growth factor predicts cognitive functions in early, drug-naive Parkinson's disease patients. *J. Neurol.* 260(2), 438–444 (2013).

108    Lim NS, Swanson CR, Cherng H-R *et al.* Plasma EGF and cognitive decline in Parkinson's disease and Alzheimer's disease. *Ann. Clin. Transl. Neurol.* 3(5), 346–355 (2016).

109    Siderowf A, Xie SX, Hurtig H *et al.* CSF amyloid {beta} 1–42 predicts cognitive decline in Parkinson disease. *Neurology* 75(12), 1055–1061 (2010).

110    Zhang J, Mattison HA, Liu C *et al.* Longitudinal assessment of tau and amyloid beta in cerebrospinal fluid of Parkinson disease. *Acta Neuropathol.* 126(5), 671–682 (2013).

111    Stewart T, Liu C, Ginghina C *et al.* Cerebrospinal fluid α-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort. *Am. J. Pathol.* 184(4), 966–975 (2014).

112    Qiang JK, Wong YC, Siderowf A *et al.* Plasma apolipoprotein A1 as a biomarker for Parkinson disease. *Ann. Neurol.* 74(1), 119–127 (2013).

113    Swanson CR, Berlyand Y, Xie SX, Alcalay RN, Chahine LM, Chen-Plotkin AS. Plasma apolipoprotein A1 associates with age at onset and motor severity in early Parkinson's disease patients. *Mov. Disord.* 30(12), 1648–1656 (2015).

114    Evatt ML, Delong MR, Khazai N, Rosen A, Triche S, Tangpricha V. Prevalence of vitamin d insufficiency in patients with Parkinson disease and Alzheimer disease. *Arch. Neurol.* 65(10), 1348–1352 (2008).

115    Knekt P, Kilkkinen A, Rissanen H, Marniemi J, Sääksjärvi K, Heliövaara M. Serum vitamin D and the risk of Parkinson disease. *Arch. Neurol.* 67(7), 808–811 (2010).

116    Ding H, Dhima K, Lockhart KC *et al.* Unrecognized vitamin D3 deficiency is common in Parkinson disease: Harvard Biomarker Study. *Neurology.* 81(17), 1531–1537 (2013).

117    Schwarzschild MA, Schwid SR. Serum urate as a predictor of clinical and radiographic progression in Parkinson disease. *Arch Neurol.* 65(6), 716–723 (2008).

118    Ascherio A, LeWitt PA, Xu K *et al.* Urate as a predictor of the rate of clinical decline in Parkinson disease. *Arch. Neurol.* 66(12), 1460–1468 (2009).

119    O'Bryant SE, Xiao G, Zhang F. Validation of a serum screen for Alzheimer's disease across assay platforms, species, and tissues. *J. Alzheimers Dis.* 42(4), 1325–1335 (2014).

120    Shi M, Bradner J, Hancock AM *et al.* Cerebrospinal fluid biomarkers for Parkinson disease diagnosis and progression. *Ann. Neurol.* 69(3), 570–580 (2011).

121    Shi M, Movius J, Dator R *et al.* Cerebrospinal fluid peptides as potential Parkinson disease biomarkers: a staged pipeline for discovery and validation. *Mol. Cell. Proteomics* 14(3), 544–555 (2015).

122    Berlyand Y, Weintraub D, Xie SX *et al.* An Alzheimer's disease-derived biomarker signature identifies Parkinson's disease patients with dementia. *PLoS ONE* 11(1), e0147319 (2016).

123    Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat. Rev. Immunol.* 2(8), 569–579 (2002).

124    Biospecimen Collection, Processing, and Shipment Manual National Institute of Neurological Disorders and Stroke Biorepository: BioSEND (2016). www.biosend.org/docs/BioSEND_Manual

125    PAR-14–259: Parkinson's Disease Biomarker Program (PDBP) Discovery Projects (U01). http://grants.nih.gov/grants/guide/pa-files/

126    Dickersin K. The existence of publication bias and risk factors for its occurrence. *JAMA* 263(10), 1385–1389 (1990).

127    *PLoS ONE*. http://journals.plos.org/plosone/

128    *eNeuro*. http://eneuro.org/

129    Lerche S, Liepelt-Scarfone I, Alves G *et al.* Methods in neuroepidemiology characterization of European Longitudinal Cohort Studies in Parkinson's disease – report of the JPND Working Group BioLoC-PD. *Neuroepidemiology* 45(4), 282–297 (2015).

130    Rocca WA, Maraganore DM, McDonnell SK, Schaid DJ. Validation of a telephone questionnaire for Parkinson's disease. *J. Clin. Epidemiol.* 51(6), 517–523 (1998).

131    Barroso MC, Esteves GP, Nunes TP, Silva LMG, Faria ACD, Melo PL. A telemedicine instrument for remote evaluation of tremor: design and initial applications in fatigue and patients with Parkinson's disease. *Biomed. Eng. Online* 10(1), 1–17 (2011).

132    Venkataraman V, Donohue SJ, Biglan KM, Wicks P, Dorsey ER. Virtual visits for Parkinson disease: a case series. *Neurol. Clin. Pract.* 4(2), 146–152 (2013).

133    NeuroBioBank https://neurobiobank.nih.gov/

134    Hall S, Surova Y, Ohrfelt A, Zetterberg H, Lindqvist D, Hansson O. CSF biomarkers and clinical progression of Parkinson disease. *Neurology* 84(1), 57–63 (2014).

135    McKeown MJ, Peavy GM. Biomarkers in Parkinson disease: It's time to combine. *Neurology* 84(24), 2392–2393 (2015).

136    Liu H, Du G, Zhang L *et al.* Folded concave penalized learning in identifying multimodal MRI marker for Parkinson's disease. *J. Neurosci. Methods* 268, 1–6 (2016).

137    Bowman FD, Drake DF, Huddleston DE. Multimodal imaging signatures of Parkinson's disease. *Front. Neurosci.* 10, 131 (2016).

## Affiliations

**Katrina Gwinn**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**Karen K David**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**Christine Swanson-Fischer**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**Roger Albin**
Neurology Service & GRECC, VAAAHS, UM Udall Center, University of Michigan, Ann Arbor, MI, USA

**Coryse St Hillaire-Clarke**
National Institute on Aging, NIH, Bethesda, MD, USA

**Beth-Anne Sieber**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**Codrin Lungu**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**F DuBois Bowman**
Department of Biostatistics, Mailman School of Public Health, Columbia

**Debra Babcock**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**Ted M Dawson**
Neuroregeneration & Stem Cell Programs, Institute for Cell Engineering, Solomon H Snyder Department of Neuroscience, Pharmacology & Molecular Sciences, Johns Hopkins University

**Richard B Dewey Jr**
Department of Neurology & Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, TX, USA

**Tatiana Foroud**
Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

**Dwight German**
Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX, USA

**Xuemei Huang**
Department of Neurology, Penn State Hershey Medical Center, Hershey, PA, USA

**Vlad Petyuk**
Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA, USA

**Judith A Potashkin**
Department of Cellular & Molecular Pharmacology, Rosalind Franklin University of Medicine & Science, North Chicago, IL, USA

**Rachel Saunders-Pullman**
Department of Neurology, Mount Sinai Beth Israel & Icahn School of Medicine at Mount Sinai, New York, NY, USA

**Margaret Sutherland**
National Institute of Neurological Disorders & Stroke, National Institutes of Health, Bethesda, MD, USA

**David R Walt**
Department of Chemistry, Tufts University, Medford, MA, USA

**Andrew B West**
Department of Neurology, University of Alabama at Birmingham, Birmingham, AL, USA

**Jing Zhang**
Department of Pathology, University of Washington, Seattle, WA, USA

**Alice Chen-Plotkin**
Department of Neurology, University of Pennsylvania, Philadelphia, PA, USA

**Clemens R Scherzer**
Department of Neurology, Harvard Medical School, Brigham & Women's Hospital, Cambridge, MA, USA

**David E Vaillancourt**
Departments of Applied Physiology & Kinesiology, University of Florida, Gainesville, FL, USA

**Liana S Rosenthal**
Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA