PARKINSON'S DISEASE

Finding useful biomarkers for Parkinson's disease

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The recent advent of an "ecosystem" of shared biofluid sample biorepositories and data sets will focus biomarker efforts in Parkinson's disease, boosting the therapeutic development pipeline and enabling translation with real-world impact.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder that manifests with motor symptoms of tremor, bradykinesia, rigidity, and postural instability, as well as non-motor cognitive and psychiatric symptoms. The second most common neurodegenerative disorder after Alzheimer's disease (AD), PD currently affects more than 4 million people worldwide, with numbers projected to double in the next few decades. Treatments are urgently needed to slow or stop the progression of PD. Of the more than 550 open studies for PD listed on ClinicalTrials.gov, only ~10% are aimed at testing disease-modifying or neuroprotective therapies. Of the existing disease-modifying or neuroprotective clinical trials, only two are in phase 3. Why is the pipeline of disease-modifying therapies so meager? Why have previous clinical trials of disease-modifying therapies been unsuccessful? Most importantly, what can we do to ameliorate this situation?

Biomarkers may represent an important tool for bolstering the therapeutic drug discovery pipeline. Biomarkers have been defined variously, but a highly inclusive definition emerged from the 2000 National Institutes of Health (NIH) Working Group on this subject: A biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (*1*).

Here, we highlight the types of biomarkers for PD that are likely to have the greatest immediate impact on the development of disease-modifying therapies for PD. We stress the urgency of finding such biomarkers in the near future, given the likely entry of several classes of mechanism-based therapies—such as those targeting the aggregation or propagation of α-synuclein or the activity of LRRK2 kinase—into early human clinical trials.

We argue that PD biomarkers that are likely to be useful in a clinical trial context should be of reasonable effect size alone or in combination (for example, demonstrate an area under the curve of >0.8) and robust, by which we mean that they must demonstrate clear reproducibility across patient cohorts. Notably, it would be preferable if the biomarker could be verified in neuropathologically proven cases of PD, given recent studies indicating that only two out of three patients seen at a first visit and given a diagnosis of possible or probable PD have PD on autopsy (*2*). Finally, practical considerations, such as cost and complexity of assays to detect biomarkers and the capability for frequent or serial testing, need to be considered early in PD biomarker discovery and development.

What should these robust, accurate, reproducible biomarkers tell us? The most

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urgent goals center on enrichment and stratification of PD patient populations, reducing clinical heterogeneity by discriminating individuals likely to have different PD trajectories. For example, objective markers that would predict whether a patient may progress faster or slower in cognitive or motor symptoms would be valuable for selecting patients for clinical trials of potential neuroprotective agents. These trials must demonstrate significant differences in the disease trajectory of individuals with PD who are treated with a disease-modifying therapy or neuroprotective agent (versus placebo). Thus, reducing the "noise" emanating from the inherent heterogeneity of disease trajectory by using biomarker-enriched PD patient populations could make such trials more efficient.

Indeed, within the first 2 years of longitudinal data collected on ~400 PD patients from the international Parkinson's Progression Marker Initiative (PPMI) (*3*), one could discern groups with distinct trajectories of motor decline (Fig. 1). Should biomarkers be found that could predict at baseline who might follow each trajectory depicted in the figure, such biomarkers could reduce heterogeneity in the PD populations selected for clinical trials, increasing the signal-to-noise ratio. Such biomarkers are not unprecedented. Imaging of dopamine transporters in the brain using SPECT (DAT SPECT) and measurements of serum urate were used for enrichment of PD patient cohorts in the SURE-PD trial that tested inosine as a disease-modifying treatment (*4*). Such an approach could be augmented in future trials, should robust biomarkers emerge from research efforts. Notably, biomarkers derived from patient biofluids are by no means the only possible markers. Indeed, as exemplified by the SURE-PD trial, other types of markers based on imaging or other modalities have proved useful. In addition, markers of target engagement may help to accelerate clinical trials. These sui generis biomarkers, however, will likely be developed in conjunction with specific therapies. Thus, they are difficult to discuss without prior knowledge of the therapeutic target, with the exception of obvious targets such as α-synuclein, glucocerebrosidase, or LRRK2.

A PIPELINE FOR BIOMARKER DISCOVERY

Underlying our preceding statements is the implicit assumption that the creation of tools enabling development of disease-modifying therapies in PD is a reachable, immediate goal, worthy of research investment. This goal is achievable because an "ecosystem" of shared data and specimen biorepositories has emerged, stressing standardized protocols for sample acquisition and storage, data analysis, and distribution. Such an infrastructure then serves as a pipeline for de novo discovery, replication of discovery findings in additional cohorts of subjects, and eventual validation of biomarker candidates. Before the advent of these shared PD patient cohorts and biobanks, investigators depended on their own ability to collect hundreds or thousands of samples for testing, preventing potential researchers lacking ready access to large clinical populations from entering the biomarker discovery arena. Within the last 5 years, however, multiple public-private efforts have laid the groundwork for investigators from both academic and industrial sectors to access well-annotated PD clinical samples. We now summarize these PD biorepositories and then place them in a potential pipeline for how they may be used to take biomarkers from concept to clinic. Whereas many PD patient cohorts exist and are summarized in an excellent recent review (*5*), here, we focus on large, multicenter cohorts with associated biofluid sample collection and clear protocols for requesting samples (Fig. 2).

DISCOVERY COHORTS: PARKINSON'S DISEASE BIOMARKERS PROGRAM AND BIOFIND

The Parkinson's Disease Biomarkers Program (PDBP, pdbp.ninds.nih.gov) was launched by the National Institute for Neurological Disorders and Stroke (NINDS) in late 2012 as a longitudinal study with biological sampling every 6 months (*6*). The PDBP features a dedicated biorepository (BioSEND) comprising DNA, RNA, and biofluid samples from >1000 individuals (>600 with PD) from multiple centers in the United States. Structural brain imaging data exist on a subsample of this PD patient cohort. An important feature of the PDBP is its associated database, the Data Management Resource (DMR), which can be directly queried by interested researchers and which serves as a portal for biosample and data requests.

Also launched in 2012, the BioFIND study (biofind.loni.usc.edu) is a collaboration between the Michael J. Fox Foundation and NINDS (*7*). BioFIND collected clinical data, as well as samples of DNA, RNA, and pellets obtained from centrifugation of whole blood from 119 PD patients and 96 neurologically healthy controls. In addition, biofluid samples of CSF, saliva, urine, and plasma were collected. BioFIND subjects came from multiple clinical centers with standardization of biosample handling protocols. Samples can be requested through the DMR.

The average duration of PD for patients in both PDBP and BioFIND is more than 5 years, and many patients are on dopaminergic medications. BioFIND does, however, feature biosample collection dates for when patients were "on" (taking) or "off" (not taking) dopaminergic medication. Given that most PD patients beyond the earliest stages are treated with daily dopaminergic medication for symptoms, the potential for medication-related confounding leading to false positives in biomarker studies is substantial. The fact that BioFIND samples have also been collected during off dopaminergic medication periods is important in addressing this concern.

REPLICATION COHORT: PARKINSON'S PROGRESSION MARKER INITIATIVE

Like PDBP, the Parkinson's Progression Marker Initiative (PPMI, www.ppmi-info.org) is a multicenter, longitudinally followed PD patient cohort with an associated biorepository (*3*). However, PPMI was designed to be a replication cohort for PD biomarkers discovered in other studies. For inclusion in PPMI's De Novo cohort, PD patients must be within 2 years of diagnosis and naïve to dopaminergic medication at study entry. De Novo cohort subjects comprise 423 PD patients and 196 normal controls, who are followed clinically. DNA, RNA, and biofluid (plasma, serum, whole blood, urine, and saliva) samples are being collected at entry and longitudinally; DAT SPECT imaging scans are obtained at entry. In addition, PPMI has recently integrated peripheral blood mononuclear cell collection, and a subset of PPMI De Novo subjects are participating in an induced pluripotent stem cell (iPSC) generation study. Imaging measures, such as functional and resting state MRI, are available from a subset of subjects, and a new Pathology Core may offer postmortem PD brain tissue samples in the future.

ADDITIONAL PD PATIENT COHORTS WITH NOTABLE FEATURES

A generic pipeline for biomarker development might involve samples from PDBP and BioFIND as discovery cohorts, with PPMI as the replication cohort. In addition, we highlight five other resources with notable features that are aiding in PD biomarker discovery efforts.

The National Brain and Tissue Resource for Parkinson's Disease and Related Disorders, housed at the Banner Sun Health Research Institute in Arizona, comprises fixed and frozen postmortem brain tissue samples from more than 150 subjects with PD and more than 250 elderly control subjects (*8*). Serum and CSF samples collected postmortem from subjects are also available.

The De Novo Parkinson (DeNoPa) study (www.denopa.de) follows subjects (159 PD patients and 110 matched healthy controls) from a single center in Germany, with a biorepository housing DNA, RNA, and biofluid (plasma, serum, whole blood, feces, and saliva) samples (*9*). Importantly, DeNoPa PD subjects enter the study earlier in the course of PD than most PD patients in the PDBP and BioFIND studies. Specifically, they are drug-naïve at enrollment, with an average disease duration of less than 2 years, thus resembling PPMI De Novo PD subjects.

The Norwegian ParkWest study (www.parkwest.no) sought to capture and recruit all incident PD cases in Western and Southern Norway in a 22-month period starting in 2004 (*10*). Two hundred sixty-five PD cases were identified, and follow-up continues. The ParkWest study is mentioned for its unusually comprehensive design and lengthy follow-up; CSF and DNA samples have been collected.

The Oxford Parkinson's Disease Centre (opdc.medsci.ox.ac.uk) houses a multicenter, prospective, longitudinal PD biomarker study (*11*). More than 1000 PD cases have been recruited, with DNA and biofluids collected.

The Harvard Biomarkers Study (neurodiscovery.harvard.edu/biomarkers-core) has collected and longitudinally phenotyped more than 2500 individuals since 2007. Notable features of this biorepository include the inclusion of more than 700 patients with early (drug-naïve and treated) PD and the accessibility of plasma, serum, RNA, and DNA samples through the PDBP DMR, as well as CSF samples for a subset of PD patients.

PRODROMAL PD PATIENT COHORTS

Pathogenic processes underlying neurodegenerative diseases such as PD and AD may be underway years or decades before onset of overt clinical features. Moreover, if the example of AD is informative (*12*, *13*), trials of disease-modifying therapies in PD could enroll presymptomatic or high-risk individuals, as well as individuals with overt PD. Thus, the development of biomarkers that can discriminate high-risk individuals who will go on to develop PD from those who will not will be important in the planning of such trials. We next discuss current efforts to enroll and follow asymptomatic individuals at high risk for PD due to genetic characteristics, for example, *LRRK2* mutation carriers, or clinical characteristics, for example, those with rapid eye movement (REM) behavior disorder (RBD) (*14*) or hyposmia (reduced sense of smell) (*15*). Samples from these cohorts represent an opportunity to develop biomarkers for pre-symptomatic diagnosis or enrichment of prodromal PD clinical trial populations. Moreover, biomarkers emerging from studies in cohorts of individuals with overt PD symptoms (for example, PDBP and PPMI) may be worth investigating in these prodromal cohorts to better understand the characteristics of these signals in early PD pathogenesis.

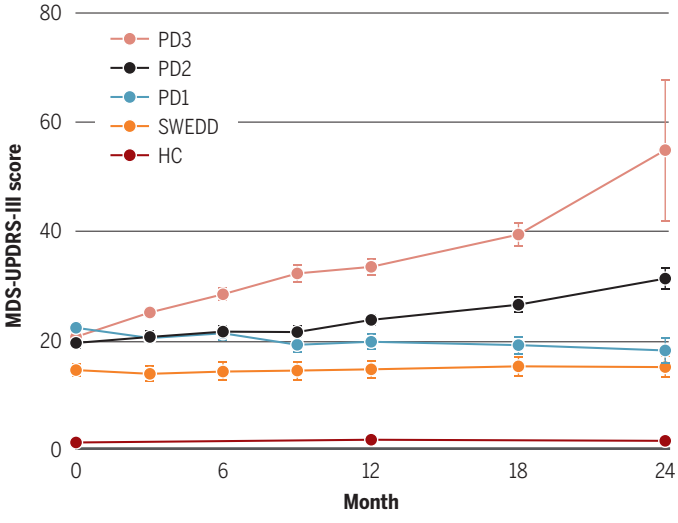

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Fig. 1. Rates of motor disease progression differ among newly diagnosed PD patients. Shown are mean and standard error of the mean for scores on the Movement Disorders Society–Unified Parkinson's Disease Rating Scale (MDS-UPDRS-III) for the PPMI cohort (*3*). PD patients in the PPMI cohort were newly diagnosed and not on dopaminergic medications. PD patients are shown stratified by MDS-UPDRS-III score: lowest tertile (PD1, blue), middle tertile (PD2, black), and highest tertile (PD3, pink). These data show that even in the absence of therapeutic interventions, newly diagnosed PD patients differ greatly in their trajectory of motor disease progression. Healthy controls (HC) are shown in maroon. SWEDD subjects (individuals who are clinically parkinsonian but may not have PD based on DAT SPECT imaging) are shown in orange.

The Parkinson's Associated Risk Study (PARS, www.parsinfosource.com) screened >10,000 asymptomatic individuals for PD risk factors, identifying 669 hyposmic subjects, 203 of whom underwent DAT SPECT imaging and DNA and biofluid collection (CSF in a subset, plasma in all) along with 100 normosmic subjects. At least 50 subjects showed <80% DAT binding in the putamen relative to age-expected norms (*16*), suggesting that many of these subjects may eventually receive a PD diagnosis.

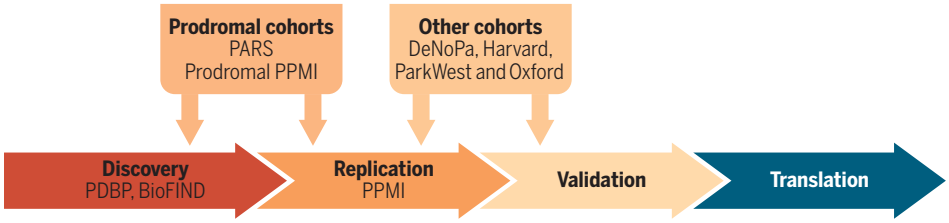
Additional cohorts within the PPMI umbrella study include asymptomatic subjects at risk for PD. For example, the prodromal subject study (prodromal PPMI or P-PPMI) includes 65 asymptomatic individuals with hyposmia or RBD and a genetic cohort study (currently enrolling) that ultimately will enroll 600 subjects with or without PD who have a PD-associated genetic mutation in *LRRK2*, *GBA*, or *SNCA*. Protocols for DNA, RNA, and biofluid sample collection mirror those for the PPMI De Novo cohort.

Current efforts to identify prodromal PD subjects rely heavily on Mendelian genetics, suggesting a potential weakness. For example, PARS and PPMI together yield <100 subjects at high risk for non-Mendelian PD, which may be too few to reliably test prodromal biomarkers.

THE ROLE OF STANDARDIZATION

Attempts to harmonize efforts among some of these studies and their biorepositories are aided by a combined Biospecimen Review Access Committee (BRAC, pdbp.ninds.nih.gov/content/application-webform) that reviews requests for samples from PDBP, BioFIND, and the Harvard Biomarkers Study.

Harmonization of access to biorepository samples streamlines the process of biomarker discovery. Standardization of other key steps may also accelerate development of biomarkers that can be translated rapidly to clinical trials. Indeed, whereas replication of a specific association between a candidate biomarker and biological process or response of interest is important, many other practical steps are needed to ultimately validate a biomarker for real-world use. For example, the experience of the AD community, which,



Cohort	#PD Patients	Stage	Biofluids				Contact
			CSF	Plasma	Serum	Other	
PDBP	>600	Established PD	√	√	√	√	pdbp.ninds.nih.gov
BioFIND	119	Established PD	√	√	√	√	biofind.loni.usc.edu
PPMI (De Novo)	423	Early PD	√	√	√	√	www.ppmi-info.org
PARS	None	Prodromal	√	√			www.parsinfosource.com
Prodromal PPMI	None	Prodromal	√	√	√	√	www.ppmi-info.org
DeNoPa	159	Early PD	√	√	√	√	www.denopa.de
Harvard Biomarkers Study	~700	Early and established PD	√	√	√	√	neurodiscovery.harvard.edu/biomarkers-core
ParkWest	~200	Incident PD	√				www.parkvest.no
Oxford Parkinson's Disease Centre	>1000	Established PD and prodromal			√		opdc.medsci.ox.ac.uk

Fig. 2. A pipeline for PD biomarker discovery and development. In the last 5 to 10 years, multiple large multicenter PD patient cohorts with associated biofluid sample collection have been assembled. Whereas, biofluid samples (for example, CSF, urine, saliva, and plasma) are collected to be used for biomarker discovery studies for some PD patient cohorts (PDBP and BioFIND cohorts), others are designed for replication of promising biomarker candidates (PPMI cohorts). The Parkinson's Associate Risk Study (PARS) and Prodromal PPMI cohorts feature asymptomatic individuals, some at high risk of conversion to manifest PD. Together, these PD patient cohorts and their associated biorepositories of patient biofluid samples form a pipeline that may facilitate the discovery and development of PD biomarkers that will aid in PD patient stratification for clinical trials testing disease-modifying therapies for PD.

through the Alzheimer's Disease Neuroimaging Initiative (ADNI), has translated both biochemical biomarkers (CSF measures of amyloid-β and tau) and imaging biomarkers (PET ligands specific for amyloid-β deposition) into clinical trial use (*12*), suggests that standardization of sample collection protocols is crucial (*17*). Also, recognizing the value that standardization may bring to biomarker studies, the European Union funded a large consortium within its neurodegenerative disease program entitled BIOMARKAPD (biomarkapd.org). Starting in 2012, with more than 48 sites in 21 European countries, BIOMARKAPD is elaborating standard operating procedures for sample collection, handling, and analysis for both AD and PD biomarkers (*18*).

One key step for standardization entails standard operating procedures for the collection and storage of biofluid samples. BioFIND, PDBP, and PPMI have harmonized standard operating procedures that can be readily accessed online. Whereas we recognize that both logistic difficulties and points of scientific disagreement may lead individual investigators and clinical sites to favor more individualized standard operating procedures, we stress the many benefits to collective action in this regard. As the harmonized BioFIND, PDBP, and PPMI standard operating procedures apply to the collection of biofluids from more than 1000 PD patients followed longitudinally across more than 50 clinical sites, we strongly urge that these detailed standard operating procedures serve as "best-practice" guidelines for the collection of biofluids for PD biomarker discovery.

A second key step in which standardization is important is in developing biomarker assays. The AD experience, especially with assays for measuring tau and amyloid-β in CSF (*19*), again provides relevant precedent. In the early stages of biomarker discovery, the best assay platform may not be clear. The community, however, will need ways to compare results obtained on alternative platforms. To that end, the PDBP developed pools of reference samples (*6*). These reference samples are simply a large set of identical aliquots obtained by pooling many samples of the biofluids in question. By including reference samples in individual assays, cross-laboratory comparisons of values obtained for a given protein assay, for example, are possible, and normalization across assays may be feasible. For a subset of biomarkers nominated beyond the discovery phase, reference standards are critical. These should be developed and shared across multiple sites.

Finally, we note that the marriage of PD biomarker discovery efforts to downstream use in clinical trials is aided by a U.S. Food and Drug Administration (FDA) program designed to facilitate this exact transition. Specifically, the FDA Biomarker Qualification Program (www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/default.htm) outlines a process by which the Center for Drug Evaluation and Research (CDER) may guide biomarker discovery scientists on practices most likely to integrate a given biomarker into drug development processes.

WHAT TYPES OF BIOMARKERS? WHAT TYPES OF PATIENTS?

With samples collected and available, what markers should we seek? Although there are myriad potential uses for biomarker discovery efforts—including the unexpected discovery of potential therapeutic pathways—we focus here on marker types most likely to accelerate the pace of disease-modifying clinical trials in PD. The average clinical trial length in PD is ~2 years, with the majority of disease-modifying trials aimed at early symptomatic PD patients. It is likely that future disease-modifying trials will also enroll the earliest symptomatic PD patients, with possible extension into the prodromal phase before disease symptoms are manifest. As a consequence, we argue that the following points should be considered in PD biomarker discovery.

The right population

If the ultimate goal is use in an early PD trial of ~2 years' length, the replication cohort should be similar, and PPMI is well suited with its longitudinal De Novo patient cohort. In earlier stages of biomarker discovery and assay development, populations with more

obvious symptoms might be used, for example, PD subjects with established disease such as those in the PDBP and BioFIND cohorts.

Large-scale discovery efforts

Despite years of effort, we do not have any biomarkers for PD that meet the criteria of acting robustly, with high sensitivities and specificities, in a way that can reduce PD patient heterogeneity in clinical trials (*20*–*22*). Many biomarker discovery studies are hampered by small numbers. For example, a systematic review of published studies investigating the utility of α-synuclein species as a PD biomarker reports that 84% of publications have data derived from sample sizes of 100 or fewer PD patients (*23*). Such studies risk "losing" discoveries due to lack of statistical power. We should be conducting discovery studies in well-powered PD patient cohorts.

Combining biomarkers

Although most existing PD biomarker studies evaluated performance characteristics of markers used alone or in very small groups, these approaches may not yield clinically useful receiver operating curve (ROC) characteristics. ROC is a method for capturing accuracies of prediction across a range of potential cutoff values. Of the PD protein biomarker studies published by PDBP investigators since 2012, only three studies reported being able to distinguish groups with an ROC of ~0.8. Two of these three studies aimed to differentiate PD patients from healthy controls or from patients with AD (*24*, *25*), and one study aimed to differentiate PD patients with or without dementia (*26*). What these three studies have in common is an approach for incorporating panels of 5 to 21 biomarkers into classifying algorithms; these algorithms, rather than individual markers, reach high classification accuracies. A strategy of constructing an aggregate measure from multiple individual markers has been fruitful in genetic studies of PD risk (*27*). Indeed, the use of markers in combination may need to span multiple modalities (for example, genetic, clinical, biochemical, and imaging-based) to maximize utility.

Biomarkers for separating PD patient subgroups

To date, most PD biomarker studies have focused on the differentiation of PD patients from healthy controls or from patients with other neurological diseases. Confirmation of diagnosis is an important goal for PD, given the difficulty posed by non-PD parkinsonian syndromes such as multiple system atrophy. These disorders, which often mimic many of the symptoms of PD, complicate PD clinical trial enrollment; the inclusion of control groups with non-PD parkinsonian syndromes is important in biomarker validation studies. Diagnosis confirmation, however, is by no means the only goal. Indeed, a more urgent need may be for markers that differentiate subgroups of PD patients that vary in rate of progression along cognitive and motor trajectories or subgroups of PD patients with important differences in pathogenesis. Unfortunately, few studies have sought to find and develop markers for differentiating PD patient subgroups.

Two lines of reasoning argue for a shift in focus for PD biomarker investigations toward inclusion of markers of differential progression. First, clinical trials aimed at testing potential neuroprotective therapies are hampered by the heterogeneity of PD progression. As shown in Fig. 1, PPMI participants not receiving any symptomatic medications differed greatly in their rates of motor progression over 24 months. The fastest third of progressors showed a substantial increase in their Movement Disorders Society–Unified Parkinson's Disease Rating Scale (MDS-UPDRS-III) score, whereas the slowest third saw essentially no change in MDS-UPDRS-III score over the same time period. Such heterogeneity makes demonstration of disease-modifying effects by potential treatments difficult, requiring the enrollment of large numbers of subjects. If one could establish biomarkers capable of reducing the variability of expected disease course in PD patient cohorts, one might substantially improve the speed and reduce the cost of clinical trial designs.

Second, there is reason to believe that biological heterogeneity may underlie the monolithic entity we call PD. We already recognize clinical subtypes of PD such as tremor-predominant and postural-instability-gait-disorder subtypes, which may also show differences in rates of motor progression and may be tagged by genetic markers such as single nucleotide polymorphisms near the *SNCA* gene (*28*). Moreover, we can now identify large subgroups of PD patients based on mutations in single genes such as *GBA* or *LRRK2*. PD patient groups stratified by *GBA* or *LRRK2* mutation status show clinical and biochemical differences (*29*, *30*), and drug development efforts aimed at these molecular targets are underway. Although few in number, biochemical markers separating PD patient groups with different rates of disease progression have also been reported. One such biochemical marker is urate in serum or plasma, lower concentrations of which are associated with increased risk for PD and faster rates of PD motor decline. Serum or plasma urate is both an outcome measure and an enrollment criterion for the phase 3 trial of inosine as a neuroprotective agent in PD (*4*).

Replication, validation, confirmation

Genetics and genomics have taught us that unbiased large-scale discovery efforts may yield promising leads. However, we have also learned that large-scale discovery efforts and particularly those generating large data sets in limited numbers of people yield many false positives. As a consequence, we need to insist on replication, validation, and confirmation of promising biomarker candidates. This requires collective change, so that we both increase incentives for and remove roadblocks to replication, validation, and confirmation.

The specific delineation of replication cohorts—such as those of PPMI—for confirmation of early results is crucial. Whereas study design features may mitigate concerns of over-fitting and false positives (for example, randomized subsampling of samples into discovery and replication sets), these features cannot entirely obviate concerns. Thus, the development of multiple independent cohorts where biomarkers can be validated is a priority.

The development of easily accessible, appropriate PD patient cohorts with standardized operating procedures serves to remove roadblocks to replication, but the problem of increasing the incentives for confirmatory studies remains. How might funding structures and publishing venues work to combat a widespread bias for the new over the confirmatory? How might we elicit the will to invest substantial time and effort in studies that definitively tell us which early hits to move forward versus those to eliminate from further consideration? These are not questions with easy answers. However, the growing recognition of widespread problems with data reproducibility in the biomedical community (*31*) argues that we cannot defer asking them.

CONCLUSIONS AND FUTURE DIRECTIONS

Biomarkers emerging from large-scale screening efforts may or may not relate to known molecular pathways involving PD. This begs the question of whether to prioritize markers for which prior knowledge suggests a clear biological link. We argue that a practical method

for weighing these considerations may be as follows. Markers with no prior known biological connection to PD (biologically agnostic markers) should move forward provided that they are consistently reproducible across PD patient cohorts and platforms, an approach that has been taken in genomics with success. As an example, we point to plasma apolipoprotein A1 (ApoA1), for which lower concentrations have consistently been found to associate with a younger age of PD onset and more severe motor impairment (*16*, *32*). Such an approach takes into account the fact that many aspects of PD pathobiology remain unknown, posing a significant challenge for top-down weighting of potential biomarker candidates, as well as potential therapeutic targets. At the same time, markers with a clear connection to PD based on prior knowledge (biologically attractive markers) should move forward provided that they are reproducible, with more attention to modifications of assay design to reduce noisy (and therefore less robust) measures. Here, we point to the many efforts that are aimed at developing CSF α-synuclein as a PD biomarker (*23*).

Regardless of the type of biomarker emerging from discovery efforts, standardization will be an important next step. We will need to make difficult decisions about assay choice and measurement conditions to make the candidate markers reproducible across PD patient cohorts and laboratories. The use of pools of reference samples early in the biomarker discovery process will help in our assessments of reproducibility across sites.

Harmonization among biomarker development efforts remains a daunting challenge. Currently, platforms, methods of processing raw data, and analysis of the processed data may all differ by investigator or biomarker. Indeed, we fully appreciate the vast leap in complexity around issues of harmonization when one moves from assaying binary, relatively robust genetic markers to assaying biochemical or other phenotypic markers that may be affected by many other factors and yield a continuum of values. There is hope, however. In the early days of RNA expression analysis, standards were developed and have been adopted widely in the field, first in the arena of microarray data. In the AD biomarker field, large-scale funding efforts with active industrial partnerships such as ADNI took a guiding role in the choice of imaging and biochemical biomarkers for which to develop specific assays. Both are viable options for moving forward, but continuing to work in an unharmonized fashion beyond the early discovery stage is likely to waste money and effort.

Finally, how might one envision the first biomarkers crossing over from research into clinical trials for PD disease-modifying treatments? Should clearly reproducible biomarkers enter the PD arena that predict, for example, the presence of PD pathology or motor disease progression, these markers should first be measured in clinical trials where PD patients are being actively enrolled. Although there may not yet be a strong enough body of evidence behind these markers to influence selection of trial participants, these markers could be ascertained in clinical trial participants and used as enrichment criteria in prespecified analyses to increase the probability of detecting therapeutic efficacy in subgroups of PD patients. Moreover, future clinical trials could be aided greatly by making data and biosamples collected during clinical trials available for independent researchers to mine. One might envision, for example, analyses of treatment effect, disease progression based on biomarker status, or inclusion of biomarkers as covariates in additional analyses, to control for some of the underlying heterogeneity that currently hampers PD clinical trials. This is a win-win scenario: Data gained in clinical trials could further strengthen our confidence in the biomarker candidates, possibly leading to their use in future clinical trial enrollment and helping to define specific phenotypes and subtypes of parkinsonism at the molecular level. Indeed, this type of "biomarker-enriched design" has been proposed for oncology trials (*33*).

We believe that these goals are achievable. Success depends on close collaboration across multiple sectors of the biomedical and drug development endeavor, including various academic groups (clinicians, pharmacologists, basic biologists, and experts in technical assay development), industry (drug developers and technology experts), government (funding sources such as NIH, standardization bureaus, and drug regulatory agencies worldwide), and private nonprofit funding agencies. These players have at times acted in distinct spheres. However, with a disease affecting so many and lacking any disease-modifying therapies, broad collaboration is essential.

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Competing interests: A.S.C.-P. consults for the Michael J. Fox Foundation and has collaborated scientifically with 23andMe. R. Albin serves on the Data Safety and Monitoring Board service for the LEGATO-HD trial (ICON/Teva), the HTTRX Trial (IONIS), and the BIIB092 trial (ICON/Biogen). R. Alcalay has consulted for Genzyme/Sanofi, Biogen, Denali Therapeutics, and Prophase. D.B. was the co-founder of Google Verily and is now an employee of Foresight. D.B. is a consultant for the Michael J. Fox Foundation. J.C. is an employee and shareholder of Biogen. D.C. consults for Caprion Biosciences. R.D. is a paid consultant to U.S. WorldMeds, Impax Pharmaceuticals, Acorda Therapeutics, Teva Neuroscience, Acadia Pharmaceuticals, Adamas Pharmaceuticals, and Lundbeck LLC. K. Merchant is the President/CEO of Chaperone Therapeutics, serves on the Scientific Advisory Board of the Michael J. Fox Foundation, and consults for AbbVie, Acumen Pharmaceuticals, Epiodyne, Lysosomal Therapeutics, Neuroinitiative, Pfizer, Sinopia Biosciences, and private equity investors. B.M. has received research grants from TEVA-Pharma, Desitin, Boehringer Ingelheim, and GE Healthcare; has received honoraria from Bayer Schering Pharma AG, Roche, AbbVie, TEVA-Pharma, Biogen, GlaxoSmithKline, and Orion Pharma; and is a member of the PPMI executive steering committee. R.S.-P. consults for Denali Therapeutics. C.R.S. has collaborated with Pfizer, consulted for Sanofi, served as Advisor to the Michael J. Fox Foundation, and is co-inventor on two U.S. patent

applications for PD biomarkers held in part by Brigham and Women's Hospital. M.v.d.B. was an employee of Genentech and is the co-founder of Vivid Genomics. D.V. is co-founder and manager of Neuroimaging Solutions LLC. D.W. is the co-founder of Illumina Inc. and Quanterix Corp. All other authors declare that they have no competing interests.

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Abstract

One-sentence summary: The recent advent of an "ecosystem" of shared biosample biorepositories and data sets will aid efforts to define biomarkers for Parkinson's disease.