## PPMI: Parkinson's Field's Answer to ADNI

19 October 2010. One of the great challenges with developing treatments for neurodegenerative disorders stems from the capricious nature of disease progression, where symptoms remain hidden or unchanged for years, perhaps even decades, despite steady accumulation of cellular wreckage within the body. Looking for ways to reliably gauge this internal damage in Alzheimer's, researchers from industry and academia launched an ambitious $64 million study in 2004. For three years, the Alzheimer's Disease Neuroimaging Initiative (ADNI) followed more than 800 participants at nearly 60 sites across North America. Analysis of this massive longitudinal dataset has drawn consensus around a handful of brain imaging and fluid biomarkers that could potentially substitute for the slower-changing cognitive measures typically used in AD drug trials (see ARF related news story and ARF ADNI series).

Now, recruitment is underway for a similar effort to identify biomarkers for Parkinson disease progression. Spearheaded and sponsored by the Michael J. Fox Foundation for Parkinson's Research, the Parkinson's Progression Markers Initiative (PPMI) is looking to enroll 400 newly diagnosed PD patients and 200 healthy controls for its study tracking three- to five-year longitudinal change in various imaging and biologic measures. With a smaller cohort than ADNI, and fewer sites (18 in the U.S. and Europe), PPMI carries an estimated price tag of $40 million. So far, GE Healthcare and Pfizer have each contributed several million dollars toward the initiative, and the Foundation hopes additional partners will chip in to jointly cover at least half the anticipated cost. Enrollment began in July, with six-month data on the initial wave of participants expected by early 2011 (see study timeline).

Just this month, an article summarizing PPMI appears in a special PD-themed issue of Biomarkers in Medicine. Anchored by a freely available overview by Michael Schlossmacher of the University of Ottawa Health Research Institute and Brit Mollenhauer at the Paracelsus- Elena Clinic, Kassel, Germany, the issue also contains individual articles on most measures included in PPMI.

The idea for PPMI emerged over many years. Motivated in part by a string of failures in clinical trials of disease-modifying agents (e.g., Parkinson Study PRECEPT Investigators, 2007), the Fox Foundation rallied leading experts for a series of workshops and discussions on the need for a large-scale PD biomarker study. "The consensus became that without more objective measures of disease progression, it was going to be difficult for other pharma companies to put their toe in the water or have a chance of success," said PPMI's principal investigator **Ken Marek**, Institute of Neurodegenerative Disorders, New Haven, Connecticut, in an interview with ARF.

The other force that propelled PPMI forward was ADNI. Based on one-year data, this public-private consortium has by and large exceeded the expectations of its organizers, who are now conducting an extension study (ADNI-GO) while also gearing up for a second phase (ADNI 2) that will focus on AD biomarkers in the earliest stages of disease (see ARF related news story). ADNI "has really set the benchmark for how to do multisite coordinated studies where data are made publicly available," **Arthur Toga**, University of California, Los Angeles, told ARF. "We had the advantage of learning from ADNI how to do things in an efficient way. We took

those lessons and applied them in PPMI." Toga heads ADNI's informatics core and now does the same for PPMI.

Like ADNI, PPMI has a "hub and spoke" design, where raw data from multiple sites get channeled to a central database and, after passing quality control, become available for access by other investigators. Toga pictures this arrangement like a bike tire, with data coming toward the center along different spokes, and then going out again. "Some spokes might connect to a quality control group, others to the biorepository core, or to a coordinating center," Toga said. "It's a communication mechanism that allows everyone to know what's going on."

For example, magnetic resonance imaging (MRI) data from a PPMI participant scanned in Boston would get sent to the study database maintained by Toga and colleagues at UCLA's Laboratory of Neuro Imaging (LONI). Once uploaded, the data are placed in quarantine, prompting automated e-mails that alert quality control (QC) teams to a new dataset in the system. By clicking a link within the e-mail, QC personnel can view the data and, if they meet specified criteria, flag it "acceptable." That releases the data from quarantine and makes them available on the study's website. Though ADNI did have a few cases where data on a patient scanned in the morning became publicly available that same afternoon, Toga said, more typically it will take a few days for data to be released from quarantine. PPMI quality control teams consist of outside parties with expertise in analyzing a particular type of data, for example, MRI, cognitive batteries, fluid assessments.

Though similar in overall structure, the informatics core plays a more central role in PPMI than it did in ADNI. Not all data from ADNI were stored in the UCLA system built by Toga and colleagues. Information on cognitive assessments, for example, was kept in a database maintained by the Alzheimer's Disease Cooperative Study (ADCS) at UC San Diego. The ADCS did not send these raw data to the UCLA system, but only shared updates based on analysis of the data. Compared to ADNI, "we have our fingers in more pies," Toga said of PPMI's bioinformatics core. "We're acting not only as the central database, but also the communication system."

One extra layer of coordination in the PPMI database arises from the study's biorepository core, which is headed by **Alison Ansbach** of Coriell Institute for Medical Research in Camden, New Jersey. Coriell is a nonprofit research institution that maintains one of the largest cell repositories for studies of aging-related diseases, including PD (see ARF iPS series). Unlike PPMI, ADNI had no bona fide biorepository division. The burden of receiving, storing and distributing biosamples fell largely to the directors of the study's fluid biomarker core—**John Trojanowski** and **Leslie Shaw**—who took this on alongside analyzing the samples and heading up their own research teams at the University of Pennsylvania in Philadelphia.

In PPMI, Trojanowski and Shaw are leading the bioanalytics team, which will validate and standardize cerebrospinal fluid (CSF) assays. However, Coriell will take care of packaging, shipping, inventory, and other nitty-gritty details that added some stress and initial chaos to ADNI (see ARF ADNI series). In particular, Coriell will provide all study sites with supplies for drawing blood, spinal fluid, and collecting urine, as well as with materials for shipping the respective samples back to Coriell for storage and distribution out to groups wanting to analyze them. "Obviously, one of

the jewels of this study is the biosamples. We have to treat them carefully," said PPMI steering committee member **Mark Frasier** of the Fox Foundation. "It's nice to have a seamless organization that, from start to finish, puts the kits together and then receives and houses the samples, all in a one-stop shop."

Though Coriell will store and distribute the specimens, sample requests will go through the LONI website, which syncs with Coriell's database to stay up to date with how much of each sample is available. Because the samples are nonrenewable, "we are looking to use them on biomarkers that already have some data," Frasier said. "We do not anticipate using the samples for novel biomarker discovery." For example, if researchers see a certain protein changing in their PD versus control samples, they could verify that discovery in a larger cohort using PPMI samples. The data should be solid but don't need to be published, Frasier noted.

All proposals will go to a panel of five to 10 biomarker experts, including one steering committee member, for review. Why the range? "We want to prevent conflict of interest where someone reviewing requests would also be wanting to use the samples themselves," said Frasier. Other considerations will be more practical—such as whether enough of the requested material is available, and whether the sample collection methods are compatible with the techniques and assays in the proposed research.

For starters, Frasier said, PPMI will focus on five potential fluid biomarkers—α-synuclein, DJ-1, Aβ, tau in CSF, and urate in blood. α-synuclein is the prime component of the Lewy body inclusions that gum up the brains of people with PD, and has been shown to be reduced in CSF of these patients (e.g., Mollenhauer et al., 2008; Tokuda et al., 2006). Mutations in DJ-1 (aka PARK7) cause early-onset PD (Cookson, 2003), and CSF levels of this protein appear to be decreased in PD as well (Hong et al., 2010). Aβ42 and tau are heavy hitters among CSF biomarkers for Alzheimer disease, and have been found to be low and high, respectively, in CSF of PD with dementia (PDD) patients as well (Mollenhauer et al., 2006). In addition, a recent longitudinal study found that low CSF Aβ42 in PD patients predicted cognitive decline over the next two years (Siderowf et al., 2010 and ARF related news story). And other research suggests an association between high plasma concentrations of the antioxidant urate and decreased PD risk (Weisskopf et al., 2007).

A big problem in the PD field, as with AD, is that different single-center CSF studies on the same biomarker don't necessarily agree with each other. Take α-synuclein, for example. **Brit Mollenhauer**, a member of PPMI's steering committee and site leader at Paracelsus-Elena-Klinik, Kassel, Germany, has worked on measuring CSF α-synuclein in PD for nearly a decade. She teamed up with Michael Schlossmacher, then at Harvard Medical School, Boston, to build and test a new ELISA for measuring total α-synuclein in unconcentrated CSF and found, quite convincingly, lower CSF α-synuclein concentrations in PD patients than in healthy controls or people with AD (Mollenhauer et al., 2008). However, others have reported elevated CSF synuclein in people with synucleinopathies (e.g., Mukaetova-Ladinska et al., 2008), and some find that CSF levels of α-synuclein do not differ between those with and without synucleinopathies (e.g., Reesink et al., 2010). "The results are not consistent," Mollenhauer said in an interview with ARF. "The problem is, [the studies] use different ELISAs, different antibodies, different standards. The variety of

measurement is huge, and you cannot compare the results." (See also ARF AD/PD story.)

Hence, one of the immediate priorities of PPMI's bioanalytics core is to conduct a side-by-side comparison of different CSF α-synuclein assays in hopes of defining a standard method for subsequent PPMI analyses. For this evaluation, four labs will each perform their particular method on a common set of samples consisting of CSF from PD patients and age-matched controls, and standard α-synuclein reference material. "We hope to start the study by the end of this calendar year," Shaw told ARF. Alzheimer disease fluid biomarker research has gone through similar trials and tribulations in recent years, and a much larger standardization and quality control initiative is well underway in that field (see ARF related news story).

The multicenter evaluation study within PPMI is an essential step toward the eventual goal of measuring CSF α-synuclein in a longitudinal cohort of newly diagnosed, drug-naïve patients. It is fairly common for PD patients with more advanced disease to take three or four different drugs, Mollenhauer noted. To rule out possible medication effects, PPMI is requiring PD patients to start the study drug-free. "We want to chart the natural course of disease without treatment," Mollenhauer said.

The scientists anticipate that recruitment for PPMI will be difficult. Though most PD patients do not go on medication immediately, many will probably start drug treatment within six to 12 months of entering the study, said steering committee member **Sohini Chowdhury** of the Fox Foundation. "There is a window before their symptoms progress to a point where they require medication, but it is definitely challenging to find these people."

In fact, PPMI chose its sites in part because of their prior success in that department. "They know how to recruit de novo patients," Chowdhury said. Mollenhauer is doing double duty in this regard. While trying to find subjects for PPMI, she and colleagues have also been recruiting for a similar German study called DeNoPa ("de novo Parkinson's"), which aims to improve early diagnosis of PD. DeNoPa participants are followed for 15 years, but come just once every two years for analyses including spinal tap, brain imaging by MRI and ultrasound, and observation in a sleep laboratory. Each visit lasts several days, during which the participant and an accompanying person (often a spouse enrolled as a control) are admitted to the clinic's research hospital situated in a historic park. PPMI follow-ups are far less extensive, but more frequent. "We'll probably just get the most motivated people for PPMI, since they get spinal taps more often," Mollenhauer said. PPMI participants have a CSF sample taken at enrollment, then at six and 12 months, and once yearly thereafter. Whereas about two-thirds of ADNI participants consented to spinal taps—far exceeding initial expectations—baseline and longitudinal CSF sampling have been made a requirement of PPMI (see ARF Webinar).

Subjects enrolling in PPMI will also get genotyped for a set of PD-associated mutations, including LRRK2, GBA, synuclein, tau, and ApoE. **Andrew Singleton** of the National Institute on Aging in Bethesda, Maryland, leads the genetics core.

PPMI enrollment kicked off in July, with 10 of 18 sites recruiting at present. Fourteen people had consented for the study as of 13 October 2010. The Kassel clinic may not

open for recruitment until early 2011, Mollenhauer said. “We are a bit behind because we first had to translate all the protocols. Now we have put them to the institutional review board, and they are discussing them. We still need to go through the radiation safety office in Germany.” None of the other European sites—Tuebingen (Germany), Innsbruck (Austria), and Naples (Italy)—have begun recruiting.

The fact that PPMI is a biomarker study, not a clinical trial, leaves investigators in somewhat of a recruitment quandary. “Patients are used to trials that test new therapies,” said **Andrew Siderowf**, steering committee member and co-investigator for the University of Pennsylvania site. “PPMI is different. The products of the study are validated biomarkers—tools for doing future clinical trials more rapidly.” Viewed in this light, PPMI may require more altruism from the patients, but on the upside, it has a much higher chance of success, he said. “When you go into a therapeutic trial, there’s an awfully good chance that whatever gets tested will be part of the process of learning, but not necessarily proven efficacious. A biomarker study, on the other hand, will produce a tool that can be used over and over again to test different sorts of therapeutics more rapidly and effectively in future trials.”—Esther Landhuis.

## PPMI: Brain Imaging To Reveal Preclinical Parkinson’s Signature?

20 October 2010. The Parkinson’s Progression Markers Initiative (PPMI) was launched in July with hopes of identifying a biomarker, or set of markers, that tracks the natural course of Parkinson disease in 400 newly diagnosed PD patients and 200 healthy controls. Modeled after the Alzheimer’s Disease Neuroimaging Initiative (ADNI), but with more initial emphasis on fluid biomarkers (see Part 1), PPMI also includes brain imaging—not only as part of the longitudinal data, but also to determine who is eligible to enroll. The study seeks people at the earliest stage of PD, namely, those diagnosed less than a year ago who have not yet gone on medication. In addition, study volunteers must have abnormal dopamine transporter (DAT) imaging at baseline. This means their brain scan needs to show evidence of dopaminergic neuron loss, as detected by single photon emission computed tomography (SPECT) imaging using a radiolabeled DAT ligand (DaTSCAN).

DaTSCAN is made by GE and has been used as a diagnostic agent in Europe since 2000, mainly to distinguish PD from other disorders with overlapping symptoms, such as essential tremor. In the U.S., the imaging agent could have gained approval by the Food and Drug Administration (FDA) in 2009, but only as a Schedule II drug, according to **John Seibyl** of the Institute of Neurodegenerative Disorders in New Haven, Connecticut. Schedule II drugs have safe and accepted medical uses, but face tighter distribution because they carry a risk of abuse. “GE decided it would be difficult for some sites to handle the burden of the extra paperwork and drug accountability associated with a Schedule II drug,” said Seibyl, who directs PPMI’s imaging core. The regulation does not consider that the radiopharmaceutical is administered at mass doses several orders of magnitude below the threshold required to approach a pharmacologic range, he noted.

Seibyl’s group did much of the early work characterizing DAT tracers and applying them as imaging biomarkers in PD. He performed those studies in collaboration with

PPMI's principal investigator, **Ken Marek**, Institute of Neurodegenerative Disorders, when both were at Yale University (Seibyl et al., 1998). Even though DaTSCAN has not yet gained FDA approval as a diagnostic, U.S. scientists can use the imaging agent for research purposes. However, delivery of the ligand to Canada is problematic, according to **Sohini Chowdhury** of the Michael J. Fox Foundation for Parkinson's Research, which is sponsoring the $40 million PD biomarkers study. As a result, Canadian sites, though well represented in ADNI, are not included in PPMI.

Seibyl and coworkers at the Institute of Neurodegenerative Disorders have tweaked DaTSCAN (aka "FP-CIT") to produce a related compound, "beta-CIT." This SPECT tracer has higher signal-to-noise ratio, which is desirable, but on the downside, it requires 16-18 hours (compared to DaTSCAN's three to four) to achieve a strong quantitative signal in the brain, Seibyl said. In practice, this means the patient can't receive the scan on the day of the injection, but must return to the clinic the next day.

Seibyl's studies with this agent indicate that SPECT imaging is sensitive enough to pick up striatal dopamine transporter abnormalities in the area corresponding to the side of the body that does not yet show clinical signs. "Imaging is very sensitive to changes in the brain even prior to motor symptoms," said Seibyl, noting that SPECT could be useful to screen for patients who are on the verge of PD. Though PPMI itself is not designed to evaluate biomarkers in at-risk populations, future phases of the study may look more closely at pre-motor stages. Similarly, the second ADNI study applies the results of the first ADNI study to place much greater emphasis on earlier, pre-dementia stages.

In the current phase of PPMI, participants will receive two types of brain imaging once a year—SPECT to gauge dopaminergic abnormalities, and diffusion tensor magnetic resonance imaging (DT-MRI). By quantifying the degree of randomness in water molecules moving through the brain, DT-MRI can pick up disturbances in the integrity of neural networks. "Water molecules move in a non-random fashion when traveling on nerve fibers," Seibyl explained. "You see more randomness in those pathways when there is a lesion. You can put a number on it." DT-MRI is being tested as an exploratory measure in eight of PPMI's 18 sites; they are Northwestern University (Chicago, Illinois), Johns Hopkins University (Baltimore, Maryland), Emory University (Atlanta, Georgia), Baylor College of Medicine (Houston, Texas), Federico II University (Naples, Italy), University of Marburg (Kassel, Germany), University of Tuebingen (Germany), and the Parkinson's Institute (Sunnyvale, California). ADNI also includes diffusion tensor imaging.

Transcranial ultrasound, a different imaging technique developed in Germany that detects areas of increased iron deposition in the brain, was also considered for PPMI. However, the data on this method have shown great variability. "Some sites are very good and very reliable. But some studies we have not been able to corroborate," Seibyl said. Ultimately, the technique was ousted because of "technical difficulties in getting consistency in a multicenter study," he said. "We thought it would be hard to achieve the rigor for standardization." Transcranial sonography and SPECT did look promising as markers in a recent study of people with a rare sleep disorder associated with elevated risk of PD and other synucleinopathies (Iranzo et al., 2010 and ARF related news story).

In the future, Seibyl sees physicians using neuroimaging to confirm predictions of elevated risk in people with sleep problems and/or lost sense of smell (aka hyposnia). The ongoing Parkinson's Associated Risk Study (PARS) aims to see whether low scores on a scratch-and-sniff smell test can reliably predict who faces elevated PD risk. Olfaction and sleep tests are included in the clinical workup done on PPMI participants. These results may be especially informative for control participants, some of whom may end up developing PD years later. "The [controls] may be the jewel in this whole study," Seibyl said. "The problem is, we've biased ourselves a little bit. By excluding people with an abnormal DaTSCAN, we've made the normal population pretty pristine." Nevertheless, if PPMI achieves its goal of validating a biomarker profile for just-diagnosed PD patients, it might be possible to "work backward and apply it to at-risk individuals to predict who is most likely to progress to PD," said PPMI steering committee member **Andrew Siderowf**, University of Pennsylvania, Philadelphia.—Esther Landhuis.