Reliable and well-validated imaging biomarkers for PD to identify individuals ‘at risk’ prior to motor symptoms, accurately diagnose individuals at the threshold of clinical PD, and monitor PD progression throughout its course would dramatically improve patient care and accelerate research into both PD etiology and therapeutics. Imaging biomarkers may be especially useful to identify individuals at risk during the pre-motor period of PD and to investigate the progression of PD during the pre-motor period. Both pathology and imaging studies have shown that PD patients have a prolonged period during which vulnerable neuronal populations are degenerating, but typical motor symptoms have not yet developed. Several studies show that about 40%-60% of dopamine neuronal markers have been lost at the threshold of motor symptoms (1, 2). While the duration of the pre-motor period is unclear, imaging and pathology data suggest that the duration is at least five years and may be as long as twenty years. Data from evaluation of post-mortem tissue has provided further evidence suggesting a characteristic temporal pattern of brain pathology in PD ascending from the brain stem to the basal ganglia and cortical regions (3). These data are consistent with the notion that there is a prolonged period during which an individual can be identified as ‘at risk’ by genetic testing, imaging evidence of neuronal degeneration, and/or early clinical signs associated with PD such as olfactory loss, REM behavior disorder, cognitive or autonomic dysfunction (4-7).

Imaging tracers targeting pre-synaptic nigrostriatal function have been the most widely used biomarker to assess early PD. Most of these studies have used either F-Dopa and/or DAT tracers to monitor dopaminergic degeneration. Specifically these imaging studies demonstrate asymmetric, putamen greater than caudate loss of dopaminergic uptake and the imaging loss correlates with worsening clinical symptoms in cross-sectional evaluation. While many studies have demonstrated that imaging markers can identify individuals with typical motor PD even with mild early disease, if imaging biomarkers are to be useful in assessing pre-motor PD, studies must move ‘backwards’ to focus on subjects ‘at risk’ but not yet diagnosed. The challenge is how to find ‘at risk’ subjects.

One approach to identifying those ‘at risk’ for PD is to assess those with one or more symptoms of parkinsonism and therefore suspected, but not diagnosed PD. The QUERY studies and the CUPS study have both examined the accuracy of DAT imaging in subjects with non-diagnostic motor symptoms (8, 9). These studies demonstrated that those individuals with suspected PD with a reduction in DAT density were at very high risk for a clinical diagnosis of PD. Similarly evaluation of nigral ultrasound also was able to distinguish among those with suspected parkinsonism, subjects likely to have an eventual clinical diagnosis of parkinsonism (10). Importantly these imaging markers cannot distinguish between idiopathic PD and other parkinsonisms like progressive supranuclear palsy, multiple system atrophy, corti-
cal basal ganglionic degeneration and diffuse lewy body disease. FDG imaging to assess metabolic anatomy may be more useful in distinguishing PD from these related Parkinson syndromes.

The use of dopaminergic imaging in clinical trials has also demonstrated the utility of imaging in very early difficult to diagnose PD. In several studies of untreated recently diagnosed PD, in vivo dopaminergic imaging has identified about 10-15% of enrolled subjects with scans in the normal range termed scans without evidence of dopaminergic deficit (SWEDD). Subsequent follow-up has indicated that those study participants with SWEDD are unlikely to have PD while those with DAT deficit are very likely to have PD (11). Therefore, in both studies of suspected PD and in studies of untreated PD (within 9 months of diagnosis) dopaminergic imaging accurately distinguishes subjects unlikely to have PD from those with an eventual clinical diagnosis of PD.

**At risk subjects – biomarkers and clinical markers**

Even prior to the development of early motor symptoms recognized in suspected parkinsonism an increasing array of early non-motor clinical markers and biologic biomarkers may reliably identify those at risk for PD and may be combined with imaging markers to further define pre-motor PD. Several clinical signs and symptoms associated with PD such as loss of olfactory function, sleep disturbances like RBD, autonomic dysfunction including constipation, and behavioral and cognitive changes may occur prior to the typical motor symptoms of PD (5, 7). These clinical features may be more accurately considered early features of PD rather than biomarkers. An emerging strategy to identify pre-motor parkinsonism has been to combine early clinical manifestations of PD with dopaminergic imaging. For example in studies of PD relatives tested for olfactory function and then undergoing dopamine transporter imaging, combining the loss of olfaction and dopamine transporter imaging density identifies a sub-group with increased risk of developing PD (11). Similarly combining RBD and dopaminergic imaging selects those subjects at risk to develop manifest motor PD. The recent and ongoing explosion of molecular genetic information uncovering several genes for PD has provided another approach to identifying an at risk and pre-motor PD population. While identified mutations generally account for a very small number of PD patients, in some populations like the Askenazi Jewish population or in regions of Spain or North African LRKK2 mutations may identify approximately 20-35% of the PD population. In addition, efforts like the International LRRK2 Consortium provide an opportunity to combine genetic, clinical and imaging assessment to clarify the pre-motor period in this genetic PD sub-set. Studies to characterize the pre-motor period in LRRK2 family members are underway. Small studies have already demonstrated that dopaminergic imaging may be abnormal in unaffected family members of PD patients gene positive for LRKK2 and other Park gene abnormalities. Imaging biomarkers for pre-motor PD may also be directed at non-dopaminergic targets. Tools may focused on a specific potential underlying pathophysiology of PD like inflammation, alpha-synuclein deposition, mitochondrial dysfunction, or protein misfolding. Recent pathological, imaging and epidemiologic data have suggested that inflammatory changes may be a risk biomarker for PD and that imaging markers of
inflammation may be an early marker of PD risk. Imaging tracers such as PK11195 targeting microglial activation have shown increased signal in PD patients. The utility of imaging markers for inflammation will depend upon improved signal to noise of current tracers and better understanding of the specificity and time course of the signal. Molecular genetics have clearly implicated increased alpha-synuclein as a risk biomarker for PD. Developing alpha synuclein assays demonstrate changes in plasma and CSF of PD patient. Alpha synuclein imaging is as yet an unfulfilled goal, but would potentially provide an ideal tool to examine pre-motor PD. Many of the early clinical manifestations of PD often preceding typical motor symptom such as loss of olfactory function, sleep disturbances like RBD, autonomic dysfunction including constipation, and behavioral and cognitive changes are most often not due to dopamine loss, but may involve pathology in norepinephrine, serotonin, cholinergic or other neuronal systems. Imaging biomarkers for these non-dopaminergic early PD associated clinical manifestation have begun to emerge. For example cardiac imaging with MIBG may identify early norepinephrine dysfunction in autonomic neurons. Both imaging and pathological studies show serotonin dysfunction in PD subjects and serotonin imaging may be a marker associated with depression. Several imaging strategies have been investigated as potential tools to identify those with early cognitive dysfunction in PD. Studies examining imaging markers such as β-amyloid burden, FDG pattern or nicotinic receptor binding have all been suggested as tools that might identify pre-motor PD dementia. Given the multiple genetic etiologies for PD already identified, the marked variability in the loss of dopaminergic markers measured by imaging at motor symptom onset and the clear heterogeneity of clinical symptoms at PD onset, it is clear that a many imaging biomarkers with a focus ranging from clinical symptoms to PD pathobiology to molecular genetic mechanisms will be necessary to fully map PD risk.

**PARS – a study to identify pre-motor PD**

The challenge of identifying pre-motor PD raises fundamental questions about the definition of PD – When does PD begin? How to incorporate non-motor symptoms and even biomarkers into the diagnostic criteria. The goal of identifying pre-motor also forces us to consider novel clinical study paradigms to screen a large population for PD and potentially to enrich the screened population for PD using one or a combination of biomarkers and early clinical markers. The Parkinson Associated Risk Syndrome (PARS) study is one example of a clinical observational study focused on establishing and following a pre-motor cohort for PD. The primary goal of this project is to develop a strategy to detect pre-symptomatic parkinsonism in a large population of individuals at increased risk for PD. This study is specifically designed to test whether combining two biomarkers of parkinsonism, olfaction and DAT imaging, in a population of first-degree relatives of PD patients provides a tool to establish an at risk PD cohort without motor symptoms of PD. We expect to collect olfactory identification testing using an already piloted mail-in UPSIT and relatives with a loss of olfaction and a subset with normal olfaction will be extensively evaluated at participating sites. This evaluation will include \[^{123}I\]β-CIT SPECT imaging to detect those with a combined loss of olfaction and a reduction in dopamine transporter density. Longitudinal clinical evaluations and a two-year follow-
up imaging evaluation will be performed to assess progression of the clinical, ol-
factory and imaging deficits and to determine whether these individuals ultimately
manifest signs of PD. The ultimate goal is to then utilize this pre-symptomatic PD
cohort to initiate studies of PD neuroprevention — to assess therapies that might
slow neuronal degeneration thereby preventing or delaying the onset of sympto-
matic PD. This study highlights both the challenge of PD ‘screening’ requiring a
very large sample size and long horizon and the potential of this approach to
inform us about the pre-motor period and ultimately therapies to prevent the onset
of PD. During the past two decades much progress has been made in identifying
and assessing PD imaging biomarkers, but as yet no fully validated biomarker for
PD is currently available. Nonetheless there is increasing evidence that molecular
genetics, focused ‘omic’ (proteomic, metabolomic, and transcriptomic) assessment
of blood and CSF, and advanced in vivo brain imaging will provide critical clues to
the pre-motor period in PD and may redefine our treatments and management of
PD patients.
Bibliography


