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Single-Photon Emission Computed Tomography and Positron Emission Tomography Evaluations of Patients With Central Motor Disorders

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Neuroimaging biomarkers in movement disorders during the past decade have served as diagnostic agents (Europe), tools for evaluation of novel therapeutics, and a powerful means for describing pathophysiology by revealing in vivo changes at different stages of disease and within the course of an individual patient's illness. As imaging with agents tracking dopaminergic function become more available, the next decade promises to enhance our clinical sophistication in the optimal use of dopaminergic imaging biomarkers for differential diagnosis, characterization of at-risk populations, guiding selection and management of appropriate treatments. The clinical role of these agents as clinical tools goes hand in hand with the development and availability of disease-modifying drugs, which carry the additional requirement for early and accurate diagnosis and improved clinical monitoring once treatment is initiated. Challenges remain in the ideal application of neuroimaging in the clinical algorithms for patient assessment and management. Further, the application of imaging to other targets, both monamineric and nonmonoaminergic, could serve a function beyond the important delineation of pathologic change occurring in patients with Parkinson's disease to suggest some role in improved phenotyping and classification of patients with Parkinson's disease presenting with different symptom clusters. New areas of focus based on the elucidation of mechanisms at the cellular and molecular level, including intense interest in alpha-synuclein and other protein inclusions in neurons and glia, have piqued interest in their in vivo assessment using scinitigraphic methods. Perhaps ultimately, treatment that is targeted to a better delineated pathophysiology-based characterization of movement disorder patients will emerge. The application of neuroimaging biomarkers to multiple ends in movement disorders provides an important model for the multiple roles diagnostic imaging agents can serve in neurodegenerative disorders; for diagnosis, for elaborating pathophysiology in patient populations, for developing new drugs, ultimately for improving clinical management.
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Parkinson's disease (PD) is the prototypic movement disorder described almost two hundred years ago by James Parkinson and is characterized by a constellation of motor and nonmotor symptoms that inexorably progress over time. Initial symptoms of PD may be very subtle, usually manifesting on one side of the body but progressing to the cardinal features of bradykinesia, tremor, disturbance of gait described in Parkinson's original monograph. Nonmotor symptoms of PD are complex and less well-characterized, including disturbances in bowel function, olfactory acuity, cognitive function, affect.[1-3] The clinical scenario is complicated by the fact that therapeutic interventions may produce a range of side effects, including dyskinesias, hallucinations, paranoid ideation. It is the nonmotor features of PD that have greatest impact on patient function and quality of life.[4,5]

Idiopathic PD is the most common movement disorder among a spectrum of diseases with common features, but different causes, prognosis, clinical course. PD accounts for approximately 80% of patients with movement disorders,

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but other clinical entities share features of PD and cause diagnostic confusion, including the Parkinson plus disorders (progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration), essential tremor, secondary parkinsonisms (vascular parkinsonism, drug-induced PD, structural/tumor, traumatic, infectious, toxin-induced, or metabolic).[1,6-10] Furthermore, other neurodegenerative disease may exhibit some motor or nonmotor symptoms confounding differential diagnosis, including dementia with Lewy bodies (DLB), Alzheimer's dementia, Huntington's, other hereditary neurodegenerative disorders. The prognosis, clinical course, optimal treatment approaches vary among these clinical entities. Hence, the need for early and accurate diagnosis is important for optimal patient care.

The subtle and insidious onset of PD contributes to this diagnostic conundrum and suggests a role for positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging in clinical assessment. The majority of patients with PD initially develop unilateral symptoms such as an upper-extremity tremor or bradykinesia that becomes bilateral with time. Abnormalities of gait are also an early symptom, whereas motor freezing, cognitive impairment, unstable posture occur later. All PD patients' symptoms progress, but at a highly variable rate. Skilled clinicians use a number of clinical features to distinguish among the parkinsonisms and other neurodegenerative disorders, including age of onset, acuteness of onset, gaze palsies, time of onset of postural instability and dysautonomias, the presence and type of tremor, timing of dementia, presence of ataxia, hallucinations, apraxia, symmetry of onset, response to dopaminergic therapies, (most important) follow-up over time.[11] Operationally, the diagnosis of PD requires the identification of 2 of 3 cardinal motor signs (tremor, rigidity, bradykinesia) and a response to L-DOPA.[12] Nonetheless, studies suggest the accuracy of clinical diagnosis is surprisingly poor in new onset patients, whether diagnosed by general practitioners or community neurologists.

Therapeutic interventions and neuroimaging evaluation in PD are based on the demonstration of degenerative changes in specific brain neuronal systems described in initial pathologic examination of PD brain nearly a century ago. In particular, the loss of dopamine-containing cell bodies whose nuclei are in the substantia nigra and project to the striatum and other brain regions is a key pathophysiologic feature of PD. This offers the rationale for treatments designed to replace dopamine (including both medications, cell implants, other biologics) or modulate the disrupted signaling pathways resulting from neuronal cell loss (eg, neural stimulators).[13,14]

Symptomatic treatment of PD is reasonably effective, although complicated in the face of progressing disease. Dopamine replacement with L-DOPA/carbidopa or dopamine agonists is the mainstay of treatment in PD. L-DOPA is converted by tyrosine hydroxylase into dopamine and, although extremely effective, must be increased over time as disease progresses. Eventually, most patients will develop dopaminergic side effects, including cycling between severe bradykinesia and dyskinetic movements as levels of brain dopamine fluctuate.[15] Further, some patients may develop debilitating nonmotor effects like hallucinations and paranoid ideation with chronic treatment.[16]

Dopamine agonists represent another common dopamine-replacement PD treatment. These drugs have less tendency to produce dyskinesias and wearing off phenomenon, but studies have consistently shown these agents to be slightly less potent in reducing motor symptoms.[17] The use of combination treatment with L-DOPA/carbidopa and dopamine agonist is sometime used to provide a longer, more controlled period of "on time" with fewer side effects.[18] The overall approach to pharmacologic treatment of PD is a matter of some contention among movement disorder specialists. Some suggest delaying the initiation of treatment to avoid the development of chronic side effects, while others advocate early initiation of therapy based on a theoretical positive impact on maintaining dopamine integrity in PD brain.[19]

Other pharmacologic treatment options are available, including anticholinergics and catechol-o-methyltransferase inhibitors. The latter are thought to enhance the amount of L-DOPA taken into brain and prolong the effect of dopamine in the neuronal synapse.[20] Surgical treatments, most notably the introduction of neural stimulators are an adjunct treatment for management of motor fluctuations in more advanced disease.

The recent emphasis on pharmacologic treatment in PD focuses on drugs that might modify the cellular mechanisms contributing to ongoing neurodegeneration. These experimental approaches can be classified as treatments to restore lost dopamine cell functions via cell transplantation or neural growth factors or neuroprotective therapies purported to retard cell loss. Neurorestorative treatments refer to cell transplant methods, brain delivery of neurotrophic factors, gene therapies for stabilizing neurons or supporting neuronal proliferation and connectivity. Neuroprotective interventions are based on the identification of the mechanisms of apoptosis and other pathways subserving cell death and devising ways to interrupt these mechanisms.[19] At present, both neurorestorative and neuroprotective treatments are in clinical investigation and do not represent proven treatment.

Despite significant progress in PD treatment during the past 3 decades, there remain critical unmet needs, including achieving an early and accurate diagnosis, assisting in the optimal therapeutic interventions that minimize the common dopamine side effects like dyskinesias while optimizing motor function, improving management of nonmotor manifestations of PD, monitoring the course of disease with agents that may retard or reverse the inevitable progression of symptoms. These clinical issues suggest an important place that neuroimaging may have in the clinical management of PD. Both PET and SPECT already play a role in clinical diagnosis where the focus has been on patients in the early stages of illness. A commercial diagnostic imaging agent is available in Europe. Yet beyond this, PET and SPECT imaging agents might serve as a biomarker of the disease process, as objective information about disease status can provide data complementary to clinical assessments, the latter complicated by the presence of symptomatic medication making accurate assessment of the native state of the disease elusive.[21]

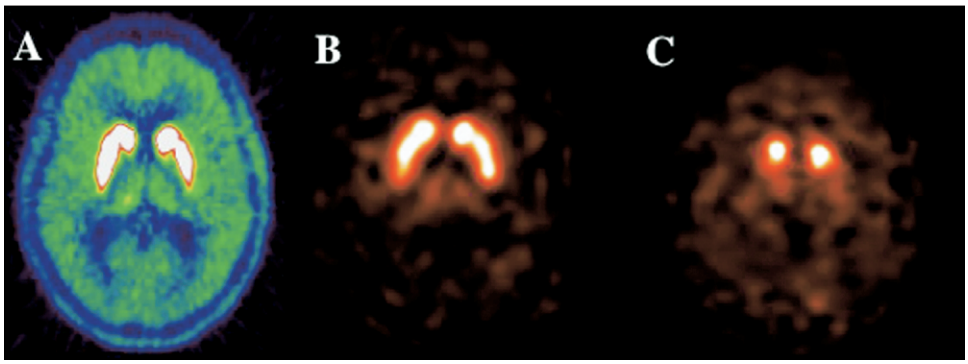

Figure 1 Presynaptic dopamine neuron imaging with ^{18}F-DOPA (A, healthy control) and the dopamine transporter agent ^{123}I β-CIT (B, healthy control; C, Parkinson's patient). Despite different molecular targets, the presynaptic PET and SPECT agents demonstrate similar patterns of uptake in healthy and diseased states and are all useful for the visual evaluation of the integrity of the dopaminergic projections to striatum.

Hence, neuoroimaging has been incorporated into clinical trials of a new generation of medications with potential for disease-modification. This application has not been without controversy as researchers struggle with imaging findings that may sometimes be discordant with clinical evaluation.[22]

Imaging Approaches to Movement Disorders

The discovery of useful radiopharmaceuticals for assessment of PD keys off the pathologic description of reduction of pigmented neurons in the substantia nigra. Hence, initial emphasis has been on imaging biomarkers of presynaptic dopamine neurons, which bear high-density targets localized in the striatum and are significantly reduced in very early disease with increasing loss as the disease progresses. These imaging biomarkers include agents targeting dopamine synthesis (^{18}F-DOPA PET), the dopamine transporter (DAT, multiple PET and SPECT agents; Fig. 1), or vesicular transporter (^{11}C-VMAT2 PET).[23,24] Each of these presynaptic markers of dopaminergic function has shown alterations in PD brain and some clinical utility as a marker of altered dopamine neuronal function. Specifically, presynaptic dopamine imaging agents demonstrate highest uptake in the striatum, an area representing the terminal projections of nigral dopaminergic neurons.

Postmortem evaluation of PD brain shows reductions in the dopamine projections to striatum with greatest involvement in the putamen relative to the caudate, a rostral to caudal gradient, significant left-right asymmetry. Presynaptic imaging markers of dopaminergic integrity mirror these pathologic findings with a highly specific pattern of loss in the striatum with greatest signal loss in the posterior putamen relative to anterior putamen or caudate consistent with pathologic data.[25,26] In newly diagnosed hemiparkinson patients who present with unilateral symptoms, all the presynaptic dopaminergic tracers show decreased uptake in the striatum on the side opposite to the symptoms. In addition, these scans demonstrate changes (to a lesser extent) on the side ipsilateral to the symptoms, the nonsymptomatic side of the body.[27] As disease progresses in PD, symptoms almost invariably become bilateral supporting the view that interrogation of dopaminergic function in PD is exquisitely sensitive, even showing pathologic reductions before the manifestation of symptoms. These studies reproducibly demonstrate 30-40% striatal signal loss in hemi-Parkinson's patients on the side mediating symptoms but also 15-25% loss on the nonsymptomatic side,[25,27,28] which suggests that significant numbers of neurons are lost before the manifestation of clinical symptoms in PD.

These very consistent findings in hemiparkinson patients underscore the potential clinical role of imaging this target in diagnosis of PD and even in the early diagnosis in patients at risk for PD based on some other high-sensitivity, but less-expensive, screening tool. Further, many of the presynaptic dopaminergic markers have been studied in longitudinal evaluation of disease progression, including, in some instances, the incorporation of putative disease-modifying agents. Such studies consistently show a striatal signal loss of about 4-11% per year in quantitative or semiquantitative measures with variability between PD subjects that mirrors the clinical variability seen in clinical assessment of symptom progression.[29-33] Nonetheless, as discussed in this article, these studies are not without controversy, especially in instances when clinical measures and imaging measures of disease progression are discordant.[18,34]

Although patterns of altered radiotracer uptake are similar among the different presynaptic dopaminergic imaging agents, the compounds evaluate different aspects of cellular function and may be useful in different ways (Table 1). For example, ^{18}F-DOPA is incorporated into dopamine neurons, where it is converted to ^{18}F-dopamine and hence is a marker of dopamine synthesis. The dopamine transporter (DAT) agents bind to a protein transporter located on the presynaptic membrane, providing a measure of transporter density as an indirect measure of nerve terminal integrity. Another useful target is the vesicular transporter that is located intracellularly on vesicles within the presynaptic nerve terminal and serves to package monoamines into vesicles for subsequent release into the synapse.

Table 1 Some Common Presynaptic Markers of Dopaminergic Function in PD

Radiotracer	^{18}F-DOPA	^{123}I FP-CIT	^{11}C-VMAT	^{123}I β-CIT	^{99m}Tc-TRODAT	^{123}I-Altropane
Molecular target	Dopamine metabolism	Dopamine transporter	Vesicular transporter	Dopamine transporter	Dopamine transporter	Dopamine transporter
Highest uptake	Striatum	Striatum	Striatum	Striatum	Striatum	Striatum
Target:background tissue ratio	Intermediate	High	Low	High	Low	Low
Optimal imaging time after injection	0 to 2 hours	3 to 4 hours	0 to 1.5 hours	8 to 24 hours	3 to 4 hours	0.25 to 0.75 hours
Comments	Has been used to directly evaluate substantia nigra	Commercial diagnostic use in Europe	New ^{18}F version in clinical trials	Used in large PD disease progression studies	High DAT:SERT selectivity	In commercial development in the US

In addition to differences in targets, dopaminergic radiotracers differ in other important ways which dictate their application in clinical and research functions. These differences include the nuclide for PET or SPECT, pharmacokinetics of brain uptake and washout, selectivity for target site, etc. For example, many dopamine transporter imaging agents have nanomolar affinity for both dopamine and serotonin transporters, although the preponderant distribution of DAT relative to SERT in the striatum makes this of limited clinical consequence. Similarly, the agent ^{11}C-VMAT2 binds nonspecifically to monaminergic neurons, but given that 95% of striatal uptake is associated with dopamine neurons, restricts the negative impact of this lack of specificity for assessing dopamine function in PD. Preclinical studies suggest that DAT may be regulated in the presence of suprapharmacologic doses of dopamine replacement drugs. However, carefully controlled test-retest clinical imaging studies in de novo PD patients now demonstrate that treatment with standard clinical doses of L-DOPA/carbidopa or dopamine agonists do not produce measurable changes in quantitative measures of DAT density. These studies suggest that imaging with these agents is reliable even in PD patients on dopamine replacement medications. This is clinically relevant in that many diagnostic evaluations using these imaging agents may be performed in PD patients on dopamine replacement treatment.

As PET and SPECT techniques for evaluating movement disorder patients have been available for almost 2 decades, a large accumulated body of studies exists demonstrating the clinical and research use of these agents in PD. These applications fall into general categories of diagnostic assessment especially to aid in differential diagnosis, monitoring disease progression, research evaluation of pathophysiology for improved understanding of the central nervous system changes underlying symptom formation and the disease process. The greatest patient experience with presynaptic imaging markers of dopaminergic neuronal function in PD are with ^{18}F-DOPA PET and ^{123}I DAT agents FP-CIT and β-CIT SPECT,[35-39] and to a lesser extent ^{11}C-VMAT2, ^{99m}Tc-TRODAT, ^{123}I-altropane, and others.[40-42]

The specific PET or SPECT radiotracer for use in movement disorders depends on the clinical or research question to be addressed by imaging. For the qualitative clinical evaluation of regional striatal signal loss to describe the presence or absence of a dopaminergic deficit in the context of making a diagnosis of PD or related disorder, all presynaptic markers have utility. Logistical factors such as radiotracer availability or the time to imaging post injection guide the radiotracer selection. If the goal is measurement of the progression of disease with serial imaging in a large clinical trial, then a qualitative, impressionistic visual interpretation is not adequate. Rather, the selection of a presynaptic dopaminergic ligand with a highly reproducible, semiquantitative, or quantitative imaging outcome measure is necessary, as well as attention to details of the imaging analysis, including pixel-wise or region of interest assessments[43-49] and incorporation of normal image databases because of the very subtle change in signal that occurs in PD brain over time.

The requirement for quantitation of the imaging signal in this context poses some difficulty as simple target (specific uptake) to background (nonspecific uptake) ratios are affected by biological and technical factors unrelated to the concentration of striatal dopamine transporters including the pharmacokinetics of uptake and washout, image processing and analysis methods, signal:noise characteristics. For example, striatal binding ratios are commonly obtained with DAT imaging agents as a semiquantitative outcome measure. These ratios are affected by the time of imaging relative to injection, the shape, size, placement size of region of interests, reconstruction, filtration, attenuation correction algorithm, biological factors including patient age, genotype, hydration status, other factors. Some radiotracers like ^{123}I β-CIT have such prolonged washout of specifically-bound striatal uptake that they closely approximate an equilibrium binding condition at the target which makes a simple ratio more quantitatively valid. Based on tracer kinetic modeling theory in such an instance the striatal binding ratio is linearly related to the density of binding sites (B_{max}).[50]

Clinical Utility of Imaging Assessments for Diagnosis and Monitoring Disease

The clinical use of imaging biomarkers in movement disorders focuses on several key issues described earlier, but at the most fundamental level should be viewed as a means to assist in the early and accurate diagnosis of PD and related disorders from conditions which may have significant phenomenological overlap.[51-54] The lack of specificity at the onset of disease leads to diagnostic confusion with disorders like vascular parkinsonism, essential tremor, drug-induced parkinsonism, Alzheimer's disease, normal aging, psychogenic etiologies.[55,56] Later in the course of illness progressive supranuclear palsy and multiple systems atrophy are most often confused with PD.[57,58]

Among primary care physicians studies suggest that almost one third of patients are incorrectly diagnosed with Parkinson's. In the very earliest cases even movement disorder specialists misdiagnosis PD 10-12% of the time. Large clinical studies using imaging offer further indirect evidence for this degree of misdiagnosis by movement disorder neurologists. In disease progression trials in de novo patients who meet operationalized diagnostic criteria for PD, serial clinical follow up and imaging reveals consistently 11-14% of subjects enrolled have scans which do not demonstrate dopaminergic deficits.[59,60] The scans have been designated "SWEDD" for "scans without evidence of dopaminergic deficit" and although the ultimate clinical diagnosis is based on clinical assessments currently, recent analysis of those patients designated as PD in these trials indicates they are clinically different from those who have abnormal baseline scans in their of lack of clinical progression, medication requirements, persistence of normal presynaptic dopaminergic imaging after 2 to 4 years without evidence of change on the scans to abnormalities typical of PD. As an example, in the REAL-PET study, a clinical evaluation of the long-term course of PD patients treated with either the dopamine agonist ropinirole or levodopa, 11% (21/193) of PD patients had normal ^{18}F-DOPA PET scans obtained at baseline and two years later.[60] Of note, the longer the duration of illness, the less likelihood that subjects will meet clinical diagnostic criteria for PD yet have normal scans (Table 2).

The implications of misdiagnosis in early PD include initiation of inappropriate treatment, sometimes resulting in unwarranted side effects, expenditures on additional medical testing, including magnetic resonance imaging or computed tomography, to rule out other conditions. Often overlooked is the fact that patients and families want a definitive diagnosis which will allow for appropriate life planning. In addition, there has been recent speculation that getting PD patients on appropriate symptomatic treatment early after diagnosis, rather than waiting until absolutely required, may have positive long-term implications on illness course.[61,62] Finally, the need for an accurate and early diagnosis is necessary to enroll patients in disease-modifying treatments when these become available.

Given these needs, how good is the performance of presynaptic dopaminergic imaging markers for differential diagnosis in patients with movement disorders? Imaging evaluations have been performed in both cohorts of patients known to have the disease and those with essential tremor, age-matched controls. Overall, these studies demonstrate outstanding sensitivity and specificity for identification of known PD.[40,63-67] Many study designs are somewhat artificial in that they do not represent the typical clinical setting in which dopaminergic imaging agents are used. In the relatively few studies performed in patients for whom there is a suspicion, but not a definitive diagnosis of PD, imaging measures also perform well with high sensitivity and specificity in diagnosis of subjects with suspected PD.[68,69] In this instance, imaging can distinguish patients with PD from those with drug induced parkinsonism, psychogenic parkinsonism, gait

Table 2 Percent of SWEDD Scans as a Function of Months Since Diagnosis in PD Studies of Progression. The Shorter the Duration of Diagnosis, the Greater the Percentage of SWEDD SPECT or PET Scans

Study	Stage of PD	Duration Dx at Baseline (months)	SWEDD/Total (% SWEDD)
Elldopa-CIT	De novo	6	21/142 (14)
PRECEPT	De novo	10	91/799 (11)
REAL-PET	De novo	9	21/186 (11)
Calm-CIT	Start of treatment	18	3/82 (4)
NIL-A –CIT	Treated, stable responder	23	3/212 (1.4)

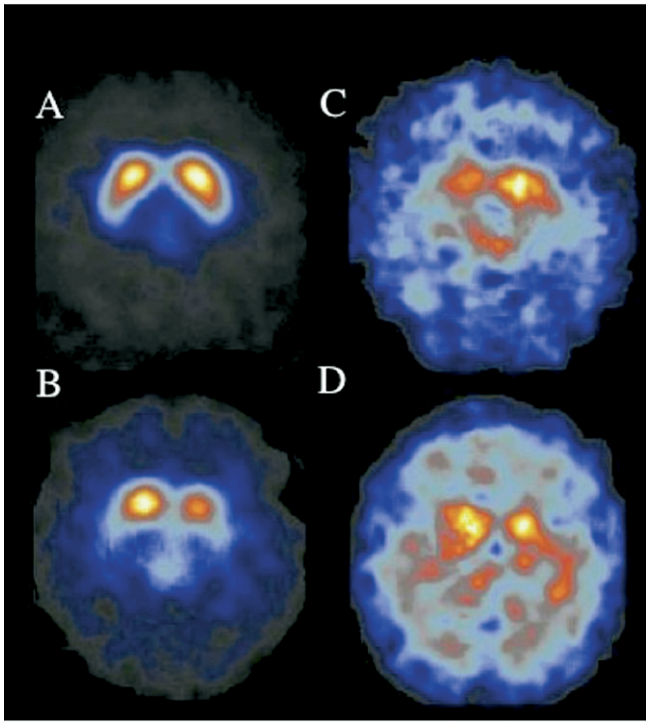

Figure 2 Transaxial SPECT images demonstrating dopamine transporter imaging in healthy control (A), Parkinson's (B) and the related Parkinson plus disorders of progressive supranuclear palsy (C) and multiple system atrophy (D). Presynaptic markers of dopamine function have been difficult to use in isolation from other clinical or imaging measures as a means to separate the parkinsonisms as all involve degenerative changes in the dopaminergic projections to the striata.

disorders mimicking PD, vascular parkinsonism, dementia. Imaging with DAT agents is used clinically to distinguish DLB from other neurodegenerative conditions producing cognitive decline, including Alzheimer's disease (AD), where DLB subjects show reductions in striatal radiotracer uptake while AD subjects do not.[70-72]

Presynaptic dopaminergic imaging is not easily used to separate among the Parkinson plus disorders, including progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration. These patients typically demonstrate significant striatal deficits on imaging with varying degrees of left-right and anterior-posterior striatal asymmetry. Unfortunately, although PD patients tend to show more asymmetric patterns of striatal uptake than in the Parkinson plus disorders, significant overlap exists between these groups, making a simple routine imaging differential diagnosis in these instances extremely difficult (Fig. 2). Some researchers have advocated the addition of postsynaptic imaging of dopamine D2/D3 receptors as providing additional information to help distinguish between PD and related disorders as PD patients show either normal or slightly increased D2/D3 dopamine receptor binding compared with mild deficits in many Parkinson plus disorders.[73-76] This observation has not translated into easily workable algorithms in the clinic.

The promised, but as yet unrealized, next generation of therapeutics in PD designed to actually reverse or retard the progression of disease, further emphasizes the need for accurate, early diagnosis to salvage as much neuronal function as possible.[77-79] This sort of treatment pushes the need to diagnose earlier, in presymptomatic at-risk patients. Studies to evaluate the feasibility of imaging at-risk populations are now ongoing, based on the sensitivity of presynaptic dopaminergic imaging to detect changes.

The high-sensitivity clinical screening tools under investigation to enrich the pool of patients who would subsequently go on to a more expensive confirmatory image procedure include olfactory function testing (known to be reduced very early in PD), neuropsychological and motor screens, genomic, proteomic, or microRNA assays.[1,80,81] Optimal algorithms are under investigation and may ultimately provide a pathway for enhancing patient identification for clinical management and further define the role of neuroimaging with PET or SPECT presynaptic dopaminergic markers.

Another potential application of presynaptic dopaminergic imaging is the serial imaging of PD patients over time to assess the progression of disease. Clinical studies have used PET and SPECT to monitor disease in this fashion as well as to evaluate potential neuroprotective drugs. Studies show a signal loss in the striatum of 4-11% per year.[60,82-84] However, these studies were done in relatively large cohorts with the advantage of adequate patient numbers and long interscan intervals to demonstrate mean changes in quantitative measures over time. The use of imaging to reliably track an individual PD patient's progressing disease is much more difficult.

Nonetheless, imaging studies have served an important role in PD drug development trials, based largely on the difficulty in designing studies to assess potential disease-modifying characteristics of medications, even in large patient cohorts, given the slow and variable course of disease progression, the time required to see a treatment effect, the confound that symptomatic medications cause in trying to assess native disease in PD patients. It is often not possible to adequately washout the symptomatic medication, which may require weeks, and ethically maintain the subject in the trial. In addition, some potential neuroprotective drugs may also provide short-term symptomatic benefit, making it difficult to distinguish disease-modification, from simple symptom reduction. Hence it is in this context that a number of large-scale disease-modification studies employ imaging as means of providing an "objective" assessment of PD status.

These studies are remarkable from the perspective of their size, duration, the issues they underscore in the interpretation of imaging data in clinical trials. These controversies may be summarized by the following questions: (1) Why are clinical measures of disease progression not well-correlated with imaging measures of progression? (2) Does medication treatment affect the presynaptic dopaminergic imaging measure? (3) Why do some patients who meet diagnostic criteria for PD have normal scans (SWEDD)?

Two large studies of the potential disease-modifying effects of dopamine agonists, the CALM-PD study and the REAL-

PET study, used ^{123}I β-CIT SPECT or ^{18}F-DOPA to follow patients who were initially treated with standardized doses of L-DOPA or a dopamine agonist for 2 (REAL-PET) or 4 years (CALM-PD).[59,60] Both studies showed very similar results despite different dopamine agonists and imaging agents; those patients initially treated with the dopamine agonists showed less reduction in the quantitative imaging measure than those PD patients treated with L-DOPA from the onset. However, although the imaging was consistent with the original study hypothesis, the PD patients treated with L-DOPA were less symptomatic than those treated with the dopamine agonist, there was only a modest correlation in the change in clinical motor ratings and the change in the imaging measure for the CALM-PD study and no correlation between clinical measures of disease progression and changes in the REAL-PET study. This lack of correlation could be explained by the inability to adequately washout the symptomatic effects of medication, or alternatively, that perhaps the treatment with either L-DOPA or dopamine agonists were producing a regulatory effect on the enzymatic processes in the ^{18}F-DOPA study or the dopamine transporter in the ^{123}I β-CIT trial. The design of these trials could not address this question directly. However, it raised questions in the neurological research community about the value and role of neuroimaging in PD trials of disease-modifying agents.

Since these studies were published, there has been significant progress on these questions. First is the observation that PD patients usually present early in their disease course with unilateral symptoms while evidencing bilateral changes on scanning, suggesting that imaging is sensitive to changes that have not yet achieved a threshold for symptom manifestation. In this case, the imaging findings in the ipsilateral striatum are completely discordant with the clinical findings.

In the CALM-PD study described previously, imaging was modestly correlated with the clinical rating scales of motor change, but only after 3 years of clinical follow-up in the trial. In another trial (PRECEPT study) recently completed in 800 PD patients evaluated for 2 years in the evaluation of a putative neuroprotective agent CEP-1347, imaging with ^{123}I β-CIT SPECT did correlate with measures of motor progression.[85] Hence, it appears that imaging measures of dopaminergic function in large trials of PD progression do track with clinical measures as long as there are adequate patient numbers and/or time in the trial to see these effects. This is true even as imaging and clinical assessments interrogate different points of the system. It is important to understand from the drug development perspective that imaging measures are not sufficient in and of themselves to demonstrate clinical efficacy of disease modifying drugs, but must be adjuncts to the clinical measures, as difficult and potentially flawed as these may be.[34]

Regarding the question of the potential regulatory effect of common dopamine-replacing drugs on imaging measures, new studies suggest this is not a factor even in the face of a complicated and contradictory preclinical literature suggesting these agents might cause regulation of the target sites like DAT. In 2 definitive studies just completed, one conducted in Europe, the other in North America, patients with PD who had never received any dopaminergic treatment were imaged with either ^{123}I FP-CIT or ^{123}I β-CIT and randomized to no treatment, L-DOPA, or a dopamine agonist at standard doses for twelve weeks, scanned again on medication, washed out of medications and scanned after eight weeks. In the preliminary analysis of North American trials with ^{123}I β-CIT there were not differences in the no treatment (n = 36), L-DOPA 600 mg (n = 38), or dopamine agonist pramipexole 3 mg (n = 38) cohorts on baseline, treatment, or washout striatal binding ratios or the percentage change in these measures between conditions among the cohorts (D. Jennings, personal communication, September 2007). These results have implications not only for clinical imaging trials of disease progression but also on the current clinical use of DAT agents in the diagnostic assessment of PD described above, as many patients with suspected PD may have already started a trial with a dopaminergic agent before their undergoing imaging as part of the routine clinical evaluation of their symptoms.

Finally, the question of the interpretation of normal scans in research subjects who meet the operational diagnostic criteria for PD has been an important source of discussion among movement disorder specialists. The long-term follow-up of these SWEDD subjects, while ongoing, points to an emerging opinion that SWEDD patients do not have PD. For example, in the PRECEPT trial of de novo patients, 66 subjects had normal baseline ^{123}I β-CIT scans. After 22 months, repeat imaging in this group demonstrated a percent reduction in striatal binding of 1.5%, compared with a reduction of 8.6% in the 634 subjects who had abnormal SPECT scans at baseline. These data are supported by the higher diagnostic uncertainty movement disorder specialists had for the SWEDD subjects than the non-SWEDD subjects (K. Marek, personal communication, December 2007).

If these conclusions regarding imaging as a sensitive tool for ensuring accurate diagnosis in those PD patients enrolled in clinical trials at very early stages of their illness are corroborated by additional data, then a strong argument can be made for using neuroimaging as part of the screening process for these studies. This is especially important given the potential misdiagnosis rate of 11-14% in these de novo patient groups and the ethicality of enrolling subjects who do not have the intended disease in long clinical trials involving exposure to novel medications. These subjects also add considerable noise to the clinical data as they do progress like the non-SWEDD subjects and hence it is possible an efficacious drug could be dropped from further development based on borderline efficacy results.

Imaging Novel Brain Targets in Movement Disorders

Although much interest has focused on the presynaptic dopamine neuron, it is useful to think of neuroimaging interrogations of dopamine systems to include presynaptic, postsynaptic, intrasynaptic function. The evaluation of intrasynaptic dopamine function refers to the demonstration several years ago that it is possible to image putative release of dopamine

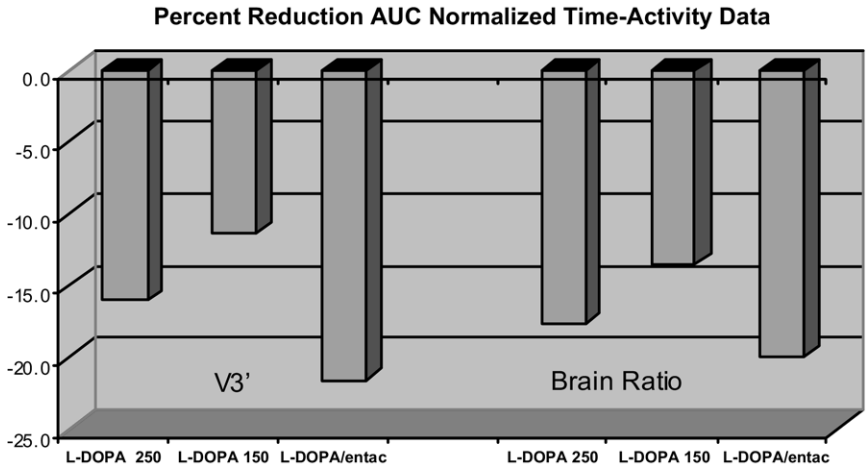

Figure 3 L-DOPA-induced reductions in 2 D2/D3 outcome measures (V3′ and striatal:cerebellar ratio) in Parkinson's subjects (n = 3) undergoing bolus plus constant infusion with ^{123}I-IBZM and serial SPECT imaging for 10 hours. This small study demonstrates 12-23% reduction from baseline in a dose-dependent fashion following oral L-DOPA administration with an enhancement of the effect by the addition of entacapone. These studies provide a putative measure of intrasynaptic dopamine in the striatum. This technique is directly relevant to the mechanism of action for many dopamine replacement therapies in PD and may be used to directly evaluate the effects of drugs in human brain.

into the synapse by measuring the amount of displacement occurring following administration of agents which increase levels of synaptic dopamine like amphetamine or L-DOPA.[86] Using a reversibly bound marker on the postsynaptic D2/D3 dopamine receptor like ^{11}C-raclopride or ^{123}I-IBZM, it is possible to evaluate a reduction of signal from these agents as endogenous dopamine competes with the labeled biomarker for occupancy at the receptor and causes reduction of the imaging signal.[87]

This technique has been evaluated in PD patients using both PET and SPECT to improve understanding of the dynamics of synaptic dopamine.[88-91] After the administration of L-DOPA 3 mg/kg intravenously in patients with PD, there is a 6-18% displacement in ^{11}C-raclopride binding. This reduction in striatal uptake putatively occurs as L-DOPA is taken up into viable dopamine neurons where it is converted into dopamine and released into the synapse, hence providing a functional measure of the status of the dopaminergic machinery in PD. In another study using this approach and ^{11}C-raclopride PET, PD patients received 250 mg L-DOPA orally and had PET studies performed at 1 and 4 hours after dosing. Those patients with significant on-off symptoms after L-DOPA showed a different pattern of reduction from those PD subjects without rapid fluctuation of motor symptoms after L-DOPA challenge. The fluctuators demonstrated a 7% reduction of striatal binding at 1 hour and return to baseline levels at the 4-hour ^{11}C-raclopride scan, whereas those without rapid fluctuations after medication evidenced protracted effects of oral L-DOPA on reduction of the striatal signal.[90]

These studies have been extended using the constant infusion method with ^{123}I-IBZM and SPECT. In this paradigm, ^{123}I-IBZM, another benzamide D2/D3 dopamine receptor agent, is intravenously administered as a bolus plus constant infusion to achieve a state of equilibrium binding at the dopamine receptor. This results in a protracted, stable baseline against which the effects of medications like L-DOPA may be studied. Serial SPECT images may be obtained over several hours taking advantage of the 13.1-hour half-life of ^{123}I. One such study using ^{123}I-IBZM administered as a constant infusion examined the effects of L-DOPA with and without the addition of entacapone, an agent purported to prolong the duration of intrasynaptic dopamine and hence ameliorate rapid motor symptom fluctuation. In a small, within-subject study in PD patients without drug-induced dyskinesia, subjects underwent three separate ^{123}I-IBZM studies with oral challenge administration of L-DOPA 150 mg, 250 mg, or L-DOPA/entcapone combination and imaged serially for ten hours after medication to evaluate the effects of drug challenge on striatal binding ratios. Similar, to the ^{11}C-raclopride studies, L-DOPA produced a displacement of radiotracer, with peak displacement of 14-19%. The concomitant of entacapone produced greater displacement with peak signal reduction of 32% and a dose–response effect on the displacement time area under curve analysis (Fig. 3).

These preliminary studies underscore the utility of examination of other aspects of dopaminergic function to improve both understanding of the changes occurring in this system as result of the disease process or chronic medication use and enhance knowledge of the effects of drugs in groups of PD patients who may respond quite differently to dopamine replacement therapies.

Other Monoaminergic Targets

Although the dopaminegic system has served as the focal point of neuroimaging investigations in PD, there is increased interest in other monamine neurochemical systems. To some extent, this derives from examination of postmortem PD brain in subjects at different stages of disease. Studies by Braak and colleagues have focused this interest based in their

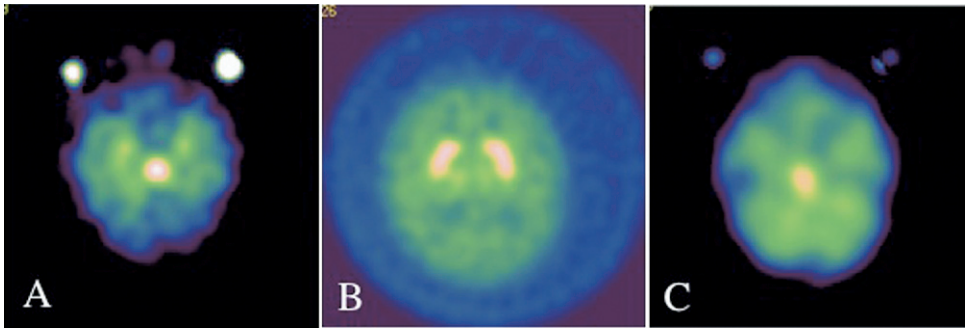

Figure 4 Representative transaxial brain images in patients with PD undergoing imaging with novel radiotracer targeting different neurochemical brain systems. Recent interest in the expansion of neuroimaging to nondopaminergic targets is fueled by post mortem studies of PD brain suggesting a complex and well-orchestrated pathologic progression originating in more primordial regions of brain with cortical extension in the course of disease. Panel (A) demonstrates evaluation of the SERT with ^{123}I-mZIENT at the level of the raphe nuclei. Areas of activity outside the brain are radioactive markers placed at the external canthi. (B) PD patient scan demonstrating acetylcholine transporter with the agent ^{123}I-IBVM at the level of the striatum, whereas (C) PD patient scan with the norepinephrine transporter agent ^{123}I-INER sliced at the level of the midbrain.

descriptions of a discrete progressive process of pathophysiologic change in brain in patients with neurodegenerative disease.[92-94] Specifically, pathologic processes originate in more primitive regions and extend over time to higher cortical regions. These observations parallel clinical reports of subtle changes in very early disease including olfactory loss and alterations in bowel function, which may be manifest some time before the appearance of the classic motor signs of PD and are associated with the functional integration of these primitive brain regions.[53,95,96] These clinical and pathologic observations serve as rationale for imaging studies of norepinephrine, serotonin, cholinergic systems, all of which project from nuclei in midbrain and brainstem regions to higher cortical region. Based on pathologic findings, it would be expected that neuroimaging markers targeting these subcortical nuclei would demonstrate reductions of specific binding consistent with pathologic loss of these neurons. The interrogation of these systems may also offer additional clues regarding the pathophysiological differences among the Parkinson plus disorders.

For example, norepinephrine neurons project diffusely throughout the brain, innervating the cerebral cortex, hippocampus, thalamus, midbrain, brainstem and cerebellum, are important in regulating autonomic function and behavioral responses such as arousal, aggression, anxiety, vigilance, affect. Serotonin cell bodies are located in the raphe nuclei and also project extensively to other subcortical and cortical regions. Alterations in norepinephrine and serotonin systems may be important in symptoms of depression and anxiety common in PD, as well as play a key role in autonomic dysfunction. Depression occurs in approximately 45% of all patients with PD and negatively impacts patient quality of life independent of the degree and extent of motor symptoms. Phenomenologically, PD depression is distinct from classic DSM-IV-R major depressive disorder[97,98] and may be among the first symptoms of the disorder, even years before motor symptoms. Many PD patients require treatment with tricyclic or heterocyclic serotonin and norepinephrine reuptake agents.

A few imaging studies have evaluated serotonin transporter (SERT) density in PD as a presynaptic marker serotonin function using PET and SPECT.[99-101] In general, these studies have involved low numbers of PD subjects with well-characterized disease and have shown alterations in SERT in PD on the order of 20-30%. This percentage has not been found by other investigators who could not detect alterations in SERT in diencephalon and midbrain regions. The relatively few number of patients studied, the resolution of imaging systems for interrogating the raphe nuclei, the nature of the control population have all been cited as confounds. Further examination of the status of SERT and other markers of serotonergic function in PD is pending.

Studies of noradrenergic function have been hampered by the slow development of a suitable ligand. A few newer agents for the norepinephrine transporter have shown promise in preclinical studies and initial human investigations. Schou and coworkers reported 2 novel PET radiofluorinated analogs of (*S,S*) ^{11}C-MeNER ((*S,S*) ^{18}F-FMeNER and (*S,S*) ^{18}F-MeNER-D_2).[102] ^{123}I-INER is a SPECT ligand related to reboxetine, which has been used in initial human studies in controls and PD patients (Fig. 4).[103] Hence, it is feasible that this system may be well-characterized in the coming years. Clinical research questions for both SERT and norepinephrine transporter targets include; (1) do PD patients exhibit changes in monoaminergic systems beyond dopamine, (2) are these changes seen early in the disorder, (3) do changes correlate with or predict those patients who have alterations in normal regulation of affect, anxiety symptoms, or postural regulation of blood pressure and other dysautonomias?

Another critical nonmotor symptom of PD that remains poorly understood and inadequately treated are the cognitive changes occurring in a significant minority of PD patients.[104-113] It is uncertain why some patients go on to develop memory loss as a later feature of illness course while others do not. In

addition, as described above the overlap of symptoms with other neurodegenerative disorders including AD and DLB may speak to some critical similarities among these diseases which ultimately can be helpful in sorting out optimal treatment. In this regard, cholinergic neuronal systems receive particular interest as medications that support cholinergic function have long been used in AD as a symptomatic treatment. Postmortem studies demonstrate cerebral cortical cholinergic deficits, including a decrease in choline acetyltransferase activity and severe losses of nicotinic binding sites, as well as cell degeneration in the basal forebrain in PD as well as in AD and DLB. In particular, a reduction in nicotinic receptor (nAChR) binding has been observed in putamen in Parkinson's disease and dementia with Lewy bodies. These findings support the idea that nicotinic acetylcholine receptor subunits are intriguing neuroimaging targets for the assessment of cognitive deficits in PD. Some post mortem reports show a decrease in the alpha4 and the alpha7 nicotinic acetylcholine receptor subunit in cortices of Parkinson patients that turns out to be similar to recent findings in Alzheimer patients. Furthermore, in vitro autoradiography investigating the distribution of 5 to ^{125}I-A-85380, a marker of $\alpha4\beta2$ nicotinic receptors, showed reductions of nAChRs in postmortem brain tissue seen in AD, DLB, PD were not apparent in vascular dementia.[114-118] These represent preliminary investigations, when translated into in vivo imaging examination holds promise for identifying phenotype based on neurochemical criteria.

Neuroinflammation and PD

Inflammation in the central nervous system is thought to play a major role in the pathogenesis of the major neurodegenerative diseases, including AD and PD from the standpoint of both initiation and continuing propagation of the degenerative cascade. In AD, neuritic plaques and tangles are the characteristic pathologic findings. Postmortem studies have revealed neuritic plaques are commonly surrounded by microglia,[119] which serve a specialized inflammatory function in the CNS. In PD, these activated microglia are present in both the subcortical and cortical regions pathologically. Evidence further suggests that PD may progress even after the precipitating cause of neuronal degeneration is removed. When microglia are activated they have been shown to produce cytotoxic substances including pro-inflammatory cytokines and reactive oxygen products, such as hydrogen peroxide and superoxide.[120-124] Cytokines can cause further activation of microglia resulting in a spiraling cycle of inflammatory change.

An important feature of activated microglia is the expression of high levels of peripheral benzodiazepine receptors (PBR) on mitochondrial membrane. PBRs are functionally and structurally distinct from central benzodiazepine receptors associated with g-aminobutric acid-regulated chloride channels. These are present at only very low levels in the normal central nervous system.[125,126]

Epidemiologic studies suggest that populations taking antiinflammatory drugs have a much reduced risk of AD and PD. This has led to several clinical drug trials to further assess the potential benefit of nonsteroidal anti-inflammatory drugs. Large, well-controlled clinical trials evaluating the benefits of antiinflammatory drugs in AD have been inconclusive, while those for PD are underway.

Neuroimaging agents like ^{11}C-PK11195, which bind PBR, have been evaluated in some studies of AD and PD. Increases in binding may be an indicator of the transition of microglia from a resting to an activated state and provide a putative assessment of the degree of inflammation as it relates to disease progression. PET imaging using ^{11}C-PK11195 has shown increased uptake, suggesting neuroinflammation in AD, PD, multisystem atrophy, cortical basal ganglionic degeneration, motor neuron disease. It is still unclear what clinical role, if any, imaging inflammation will have in PD. From a research perspective, it would be useful to assess changes in PBR as a function of treatment. It remains to be seen whether such changes will reflect alterations in rate of disease progression. In fact initial ^{11}C-PK11195 studies suggest inflammation may be noted at disease onset, but only small studies have been completed to date evaluating serial imaging of PBR in patients.

Conclusion

Neuroimaging biomarkers in movement disorders during the past decade have served as clinically available diagnostic agents (Europe), tools for evaluation of novel therapeutics, powerful means for describing pathophysiology by revealing in vivo changes at different stages of disease and within the course of an individual patient's illness. As imaging with agents tracking dopaminergic function become more available, the next decade promises to enhance our clinical sophistication in the optimal use of dopaminergic imaging biomarkers for differential diagnosis, characterization of at risk populations, guiding selection and management of appropriate treatments. The clinical role of these agents as clinical tools goes hand in hand with the development and availability of disease-modifying drugs, which carry the additional requirement for early and accurate diagnosis and improved clinical monitoring once such treatment is initiated.

Challenges remain in the ideal application of neuroimaging in the clinical algorithms for patient assessment and management. Further, the application of imaging to other targets, both monamineric and nonmonoaminergic, could serve a function beyond the important delineation of pathologic change occurring in PD to suggest some role in improved phenotyping and classification of PD patients presenting with different symptom clusters. New areas of focus based on elucidation of mechanisms at the cellular and molecular level, including intense interest in alpha-synuclein and other protein inclusions in neurons and glia piques interest in their in vivo assessment using scinitigraphic methods. Perhaps ultimately, treatment targeted to a better delineated pathophysiology-based characterization of movement disorder patients will emerge. The application of neuroimaging biomarkers to multiple ends in movement disorders provides an important

model for the multiple roles diagnostic imaging agents can serve in neurodegenerative disorders; for diagnosis, for elaborating pathophysiology in patient populations, for developing new drugs, ultimately for improving clinical management.

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