Advances in the Role of Neuroimaging to Monitor Disease Progression in Parkinson's Disease

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Abstract

Since structural imaging has generally failed to demonstrate consistent abnormalities in Parkinson's disease (PD), from an imaging perspective, the diagnosis has typically been based upon the demonstration of impaired striatal dopamine (DA) function. Radiotracer imaging techniques such as positron emission tomography (PET) and single photon emission computerized tomography (SPECT) allow the *in vivo* assessment of nigrostriatal DA function as well as regional cerebral blood flow, glucose metabolism, and functional connectivity, and changes in these measures have been used to infer disease progression. Pre-synaptic radiotracer imaging (RTI) has shown that striatal dopaminergic hypofunction follows a negative exponential pattern with the fastest rate of decline in early disease. Moreover, while striatal subregions remain differentially affected throughout the course of disease, with the posterior putamen affected more than anterior structures, the rate of deterioration is similar in all subregions. However, although functional imaging is undoubtedly a very useful tool in the assessment of PD progression, various studies have shown discordance between clinical progression of PD and nigrostriatal degeneration estimated by PET or SPECT. Therefore, considerable caution is warranted in the interpretation of imaging findings. While a potentially invaluable complement in assessing the severity of dopaminergic dysfunction, functional imaging is not a substitute for clinical assessment and other objective measures of PD severity, and cannot be currently considered a biomarker for progression of PD.

Keywords

Neuroimaging, positron emission tomography, progression, Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder and affects more than one in 100 people over the age of 65.[1] The cardinal clinical features of PD are bradykinesia, resting tremor, rigidity, and postural instability.[2] The pathophysiologic hallmark of PD is degeneration of the dopaminergic neurons of the substantia nigra leading to striatal dopamine (DA) deficiency.[3] Symptoms of PD appear when 80 % of striatal DA, or 50 % of nigral cells, are lost.[4,5] While there has been a recent emphasis on non-dopaminergic aspects of PD,[6] DA replacement therapies, including levodopa and DA agonists, are very effective in treating the cardinal features of the disease. With the possible exception of recent evolving techniques, structural imaging has generally failed to demonstrate consistent abnormalities in PD. Thus, from an imaging perspective, the diagnosis has typically been based upon the demonstration of impaired striatal DA function and changes in these measures have been used to infer disease progression. Radiotracer imaging (RTI) techniques such as positron emission tomography (PET) and single photon emission computerized tomography (SPECT) allow the *in vivo* assessment of nigrostriatal DA function, as

well as regional cerebral blood flow, glucose metabolism and functional connectivity. Molecular imaging techniques may also provide insights into pathophysiologic mechanisms such as neuroinflammation or abnormal protein deposition. However, considerable caution is required in the interpretation of the findings and while a potentially invaluable complement in assessing the presence and severity of dopaminergic dysfunction, these techniques are not a substitute for careful clinical assessment and other objective measures of PD severity.

Neuroimaging of the Nigrostriatal System
Dopamine Synthesis, Storage and Re-uptake

DA is synthesized by the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (L-dopa), which is in turn decarboxylated to DA by L-aromatic amino acid decarboxylase (AADC). Exogenously administered L-dopa crosses the blood–brain barrier via the large neutral amino acid transporter. The vesicular monoamine transporter type 2 (VMAT2) pumps both newly synthesized and recycled DA into presynaptic vesicles. Vesicular storage helps to maintain the molecular

integrity of neurotransmitters by preventing their catabolism to potentially toxic compounds. Axonal depolarization leads to release of DA into the extracellular space, leading to its interaction with pre- and post-synaptic DA receptors. The molecular effects of DA are terminated by conversion via methylation and oxidative deamination to homovanillic acid, but primarily by re-uptake into presynaptic terminals from the synaptic cleft. The membrane DA transporter (DAT) mediates this re-uptake, following which DA is either recycled into storage vesicles or converted to inactive metabolites.

Presynaptic Imaging

There are three basic approaches to the assessment of presynaptic dopaminergic integrity using radioligands to measure various aspects of the striatal processing of DA.

- 6-[18F]-fluoro-L-dopa (FD) is used as a marker to monitor the uptake and decarboxylation of FD to fluorodopamine (FDA), and the subsequent storage of FDA in synaptic vesicles. It has been extensively characterized and is widely regarded as the 'gold standard' for assessing the integrity of the nigrostriatal DA system. FD uptake correlates with nigral cell counts and with striatal DA levels in humans[7] and in non-human primates with MPTP-induced parkinsonism.[8] It correlates somewhat with the clinical severity of PD, particularly with bradykinesia, but not with tremor.[9] FD uptake is usually determined over 90–120 minutes following tracer injection, during which time tracer uptake is unidirectional in the normal brain, reflecting the trapping of FDA in synaptic vesicles.[10] Prolonged scan times of up to four hours (during which there is tracer egress and subsequent metabolism to 18F-HVA) can be used to assess effective DA turnover, which is increased in early PD.[11] Effective DA turnover (EDT) as measured by FD PET correlates well with classical post-mortem measures of DA turnover (i.e. the ratio of DA metabolites to DA).[12] 6-[18F]-fluoro-m-tyrosine (FMT) is also taken up and decarboxylated by DA neurons in a fashion analogous to FD. FMT, which is not as widely available as FD, has the advantage of a higher signal-to-noise ratio than FD (because it is not a substrate for catechol-O-methyltransferase [COMT]) and may be useful for assessing the degree of DA denervation as well as the response to treatment interventions.[13–15] However, because it is trapped in the striatum (although not in synaptic vesicles), it is not useful for the study of DA turnover.
- The DAT is the principal mechanism responsible for the termination of DA activity in the normal brain and can be labeled for PET using a variety of [18F] or [11C] labeled antagonists and for SPECT using a variety of tropane (cocaine-like) derivatives labeled predominantly with [123I], but also potentially with [99mTc]. The DAT is found exclusively in DA axons and dendrites[16,17] and its levels correlate with striatal DA concentrations[18] and with the clinical severity of PD.[19,20] The reproducibility of scan results within subjects is also acceptable.[21–23] As the DAT is downregulated in early PD, the levels may be reduced below those expected for the degree of dopaminergic nerve terminal loss and it is accordingly a sensitive marker of impairment. The potential sensitivity of the DAT to pharmacologic regulation and to compensatory alterations has also been raised as a theoretical concern in the interpretation of the findings (particularly in the case of potential disease-modifying

therapies; see more below), but the clinical significance of this potential limitation is unclear.
- [11C]-dihydrotetrabenazine (DTBZ) can be used to determine the VMAT2 density. There are two forms of VMAT expressed in humans: VMAT1 is found in the adrenal glands, while VMAT2 is expressed exclusively in the brain. VMAT2 is a 515-amino acid protein responsible for the uptake of intra-cytoplasmic monoamines into synaptic vesicles. VMAT2 is not specific for DA, but is responsible for the packaging of all monoamine neurotransmitters; however, in the striatum, more than 95 % of VMAT2 binding is to DA nerve terminals. In rats, striatal VMAT2 binding as measured with [3H]-methoxytetrabenazine correlated with SNc neuronal density.[24] VMAT2 is thought to be less subject to pharmacologic regulation and compensatory changes than other markers of dopaminergic function[25,26] and this may in theory make it a more suitable approach to assess DA innervation. However, recent evidence suggests that VMAT2 binding may in fact be affected by marked changes in the vesicular concentration of DA.[27–30]

The interpretation of dopaminergic scans needs to take into consideration the fact that tracer uptake is not a direct measure of dopaminergic neuronal density. Early PD is characterized by relative increases in FD uptake compared to the degree of denervation as assessed by DTBZ PET, probably reflecting compensatory upregulation of AADC activity.[31] Hence, FD uptake may underestimate the degree of dopaminergic denervation, particularly in early disease. On the other hand, VMAT2 expression per existing DA terminal is thought to be relatively resistant to regulatory changes resulting from denervation and pharmacotherapy. DTBZ binding correlates well with presynaptic vesicle density and hence, in turn, reflects the nerve terminal density, although it is subject to competition from cytosolic DA and extensive depletion of vesicular DA may therefore lead to apparent elevation of VMAT2 binding.[32,33] Although DAT binding might be expected to reflect DA terminal density, the DAT is down-regulated in early PD as a compensatory change[31] and may be further influenced by pharmacotherapy and age.[34,35] Therefore, DAT binding may tend to overestimate nigral cell loss and scan interpretation must include some correction for age.

Parkinson's Disease-related Spatial Covariance Pattern

While imaging with tracers specific for dopaminergic function is the most obvious approach to assessing nigrostriatal integrity, another strategy is to assess energy metabolism using 18F-fluorodeoxyglucose (FDG). By mapping glucose metabolism at a voxel level, this imaging approach provides a measure of regional synaptic activity and the biochemical maintenance processes that dominate the rest state. The effects of localized pathology on these cellular functions can alter functional connectivity across the entire brain in a disease-specific manner. While this approach is less neurochemically specific than scans performed with dopaminergic agents, PD is associated with the expression of an abnormal metabolic pattern characterized by increased pallidothalamic and pontine activity, and concurrent relative metabolic reductions in the cortical motor and association regions. The PD-related spatial covariance pattern expression is highly reproducible[36] and in addition to the accurate discrimination between patients with PD and healthy volunteers, this network measure was useful in the differential diagnosis of classic PD and atypical forms of parkinsonism.[37] Substantial evidence links the PD-related spatial covariance pattern to

the motor manifestations of the disease. The activity of this network is associated with standardized motor ratings[38] and spontaneous firing rates of neurons in the motor pallidum.[39] Moreover, PD-related spatial covariance pattern activity can be modulated by therapeutic lesioning or deep brain stimulation of the motor pallidum and the subthalamic nucleus.[38,40] The reduction in network activity induced by these interventions is associated with the degree of post-operative motor benefit seen.

Postsynaptic Imaging

D1 and D2 DA receptors can be evaluated using a variety of radiotracers. [18F]-fallypride, [11C]-FLB-457 (PET) and [123I]-epidepride (SPECT) are ultra-high affinity DA receptor antagonist radioligands, which allow quantification and visualization of low density DA extrastriatal D2/D3 receptors as well as striatal receptors.[41] The radioligands [11C] raclopride (RAC) (PET) and [123I] IBZM (SPECT) are widely employed to assess striatal DA receptor availability. As these ligands have a lower affinity for D2/D3 receptors, quantification of extrastriatal receptors is not possible.[42] However, RAC and IBZM are subject to competition from endogenous DA for *in vivo* binding to D2 receptors and changes in binding can therefore be used to infer alterations in synaptic DA concentration. Tracer binding is also influenced by age and to a lesser extent, the stage of PD and DA replacement therapy (DRT). While increased tracer binding is observed in the more affected putamen in early PD,[43,44] advanced PD and chronic DRT result in normalization of binding in the putamen and decreased binding in the caudate.[43,45] In early PD, increased D2 binding has also been demonstrated using [11C]-N-methylspiperone.[44] Unlike RAC, the binding of this ligand is not thought to be subject to competition from endogenous DA and the findings therefore suggest that increased binding of D2 ligands to putaminal D2 receptors in early PD reflects receptor upregulation, in addition to increased receptor occupancy resulting from endogenous DA deficiency. In contrast, D1 binding as assessed by [11C] SCH23390 and PET is normal in PD,[46] but may be decreased in conditions characterized by loss of striatal neurons, such as multiple system atrophy.

Assessment of Disease Progression With Positron Emission Tomography Imaging

FD uptake in early PD is most severely decreased in the dorsal part of the caudal putamen but significant decreases can be seen throughout the striatum. Several series have now demonstrated that the loss of striatal FD uptake occurs more rapidly in PD patients than in age-matched controls.[47–50] In these studies, the mean annual rate of decline in FD uptake in PD patients has been reported to range from 8 to 12 % in the putamen and 4 to 6 % in the caudate, whereas the annual decline in normal volunteers is lower than 1 % (0.5 and 0.7 % in the putamen and in the caudate, respectively).[50] In patients with unilateral disease, the clinically unaffected putamen is still abnormal, in keeping with subclinical loss of dopaminergic function.[31,51,52] An anterior–posterior gradient of dopaminergic dysfunction has been demonstrated in the putamen, with side-to-side asymmetry in tracer binding between the more and less severely affected striatum. As disease progresses, the anterior-posterior gradient is maintained, suggesting a similar relative rate of decline throughout the putamen, while the degree of asymmetry between less and more severely affected putamen diminishes.[53,54] While striatal sub-regions remain differentially affected throughout the course of the

disease, the rate of deterioration is similar. It has been suggested that the absolute rate of decline does not vary between different regions of the striatum. In a five-year longitudinal study with FD PET, Nurmi et al. evaluated the annual rate of decline of tracer uptake in different striatal subregions.[50] They found a 10.3 % annual reduction in 18F-dopa uptake in the posterior putamen and an 8.3 % reduction in the anterior putamen, compared to baseline values. Caudate nucleus showed a 5.9 % annual reduction. The absolute rate of decline however, was similar in all striatal subregions. An analogous study was performed in 31 untreated patients with early PD.[55] Patients were studied with FD PET twice, at the time of diagnosis and two years later. Results from this study also indicate a similar rate of progression between the subregions of the striatum.

Taken together, these findings support the notion that while factors responsible for disease initiation affects striatal sub-regions differently, disease progression may be due to non-specific mechanisms such as oxidative stress/free radical elaboration, excitotoxicity, mitochondrial damage, inflammation, etc.[56–58] that affect striatal sub-regions to a similar degree.

Nurmi et al.[59] have also used 18F-CFT PET to investigate DAT loss in striatal subregions in early PD patients. They found that the decline in tracer uptake was not significantly different between anterior and posterior putamen. When the rates of progression were calculated compared to the normal control mean, the caudate had the highest rate of progression (5.6 %), followed by the anterior putamen (5.3 %), and then the posterior putamen (3.3 %). However, the absolute decline in 18F-CFT PET uptake was greater in the less affected putamen. This finding is in keeping with a non-linear—likely exponential—rate of progression that should be slower in the more affected posterior putamen, where the disease is more advanced at baseline.

Analysis of longitudinal data with multitracer PET in PD at our center shows that at symptom onset, there is a substantially greater loss of putamen DTBZ binding in younger, compared with older, PD patients. Additionally, the rate of progression of putaminal TBZ binding loss in younger patients is slower compared to older ones, and the estimated presymptomatic disease phase spans >2 decades in younger patients, compared to one decade in older cases. This suggests that younger PD patients' symptoms progress more slowly and are able to endure more damage to the dopaminergic system before the first motor symptoms appear, both possibly due to more efficient compensatory mechanism.[60]

Autopsy studies in PD have shown a 45 % decrease in nigral cell counts during the first 10 years of PD, 10 times greater than the loss associated with normal aging, with a tendency to approach the normal age-related linear decline in the later stages.[5] A number of PET studies support this non-linear pattern of nigral cell loss.[53,54] Dopaminergic hypofunction in the putamen, as demonstrated by decline in FD uptake, is more rapid in early disease than in later stages, supporting the hypothesis of negative exponential decline.[54,59,61]

Estimation of preclinical duration in PD based on FD PET studies varies according to the model that is used as well as other technical factors, but is approximately six years,[49,62] with estimated losses ranging from

approximately 30[49,62] to 55 %[31] of normal putaminal FD uptake at the time of symptom onset, in general agreement with post-mortem studies.[5,49,62] A recent study based on our analysis of longitudinal DTBZ data suggests that the preclinical interval varies depending on the age of onset (longer preclinical duration in subjects with a younger age of onset) and that for DTBZ, the preclinical duration is approximately 17 years (for onset age of 53), but that at the same age of onset, the calculated preclinical durations are shorter when other tracers are used (13 years for DAT binding; six years for FD uptake, in keeping with other literature).[60]

The sensitivity of imaging to detect preclinical dopaminergic dysfunction has been demonstrated in a number of high-risk groups, including asymptomatic individuals exposed to MPTP[63] and unaffected twins whose identical co-twins are affected.[64] DA dysfunction has been demonstrated in clinically unaffected mutation carriers from families with LRRK2 mutations[65] and progression in these families has been associated with further decline in dopaminergic tracer uptake, with clinical involvement appearing to correspond to progression of impaired FD uptake.[66] Interestingly, abnormalities of DA turnover can be seen very early in LRRK2 mutation carriers, many years prior to the expected age of disease onset, and earlier than changes in other markers of DA function.[67] Changes in FD uptake have also been reported in asymptomatic parkin and PINK1 heterozygotes[68,69] although the significance of this is still not resolved.

Changes in metabolic network activity can also be used to track PD progression.[70] PDRP activity has been shown to increase linearly with disease progression. Disease progression was associated with increasing metabolism in the subthalamic nucleus (STN) and internal globus pallidus (GPi), as well as the dorsal pons and primary motor cortex. On the other hand, declining metabolism was found in the prefrontal and inferior parietal regions with advancement of disease. PDRP expression was elevated at baseline relative to healthy control subjects, and increased progressively over time. Changes in PDRP activity correlated with concurrent declines in striatal DAT binding and increases in motor ratings. However, the PDRP is expressed in the asymptomatic striatum in patients with unilateral disease and progresses symmetrically and in parallel with the symptomatic striatum, with no difference in values between the two sides. A relatively short preclinical period is indicated by network analysis of metabolic imaging data, in which the dissociation of the normal relation between metabolic activity and age occurred about five years prior to the onset of PD symptoms.[71]

Structural Neuroimaging to Assess Disease Progression

DTI is a promising new MRI technique to assess gray and white matter microstructure integrity by measuring regional fractional anisotropy (FA), a measure of the directional diffusivity of water. In a recent DTI study of 14 PD patients and 14 normal individuals,[72] PD patients as a group showed reduced FA values in the SN compared with controls. Also, all individual PD patients were distinguished from all healthy controls on the basis of their FA values in the caudal region of interest with 100 % sensitivity and specificity. If confirmed in larger cohorts of PD patients, these findings would suggest that DTI may be a useful tool for the diagnosis of PD. It remains to be established, however, whether serial assessments of nigral FA may provide a non-invasive biomarker of disease progression.

The substantia nigra is rich in iron and it has been suggested that this may contribute to disease pathogenesis in PD. In 26 patients with early PD and 13 control subjects studied with 3-tesla MRI and transverse relaxometry, Martin et al. found that midbrain iron content in the lateral substantia nigra pars compacta was higher in early PD patients, and correlated with disease severity.[73]

A multimodal magnetic resonance imaging study of subcortical gray matter structures using 3-T MRI with T2*-weighted, whole-brain T1-weighted and diffusion tensor imaging scans was recently reported in 30 patients with PD and 22 controls.[74] Patients with PD displayed significantly higher R2* values in the substantia nigra (in keeping with the report of Martin et al.), lower fractional anisotropy values in the substantia nigra and thalamus, and higher mean diffusivity values in the thalamus. Voxel-based analyses confirmed these results and, in addition, showed a significant difference in the mean diffusivity in the striatum. The combination of the above three markers was sufficient to obtain a 95 % global accuracy (area under the receiver operating characteristic curve) for discriminating patients with PD from controls.

Transcranial ultrasound can be used to demonstrate increased echogenicity in the PD midbrain, also thought to be reflective of increased iron content.[75,76] However, echogenicity does not seem to change with disease progression and this modality is accordingly unlikely to be helpful for following disease progression.[77]

Although promising, much further work is needed with structural imaging techniques before they can be considered more useful in PD both as potential biomarkers of the disease or for the assessment of disease progression.

Disparity Between Clinical and *In Vivo* Measures of Disease Progression

RTI has been used as an *in vivo* biomarker to assess the effect of treatment on disease progression in various clinical trials. These studies include the Comparison of the agonist pramipexole with levodopa on motor complications of Parkinson's disease (CALM-PD) study[78] which used β-CIT SPECT (a measure of DAT binding) to compare the effects of L-dopa versus pramipexole in early PD, the REAL-PET study,[79] which compared the use of ropinirole and L-dopa in de novo PD patients using F-DOPA PET as the primary outcome measure, the Earlier versus later levodopa in Parkinson disease (ELLDOPA) study[80] in which the effects of L-dopa on clinical progression of PD were studied, and βCIT SPECT was included, and studies on fetal nigral transplantation with FD PET as a secondary outcome measure.[81–84] The effects of intraputaminal glial cell line-derived neurotrophic factor (GDNF) on clinical and imaging end-points have also been reported.[85]

All the above studies found heterogeneity in results between clinical outcome and estimated disease progression, as determined by SPECT or PET. Imaging findings suggested a slower rate of disease progression with pramipexole in the CALM-PD study[34,78] and with ropinirole in REAL-PET.[79] However, the clinical improvement, based on the Unified Parkinson's Disease Rating Scale (UPDRS), favored the L-dopa treatment group. In the ELLDOPA study,[86] the L-dopa treatment group had a

slower rate of clinical progression compared with the placebo group when clinical assessments were performed after a wash-out period of two weeks. Although this probably reflects inadequate washout of symptomatic effects, a more rapid rate of decline in DAT binding was noted with β-CIT imaging in the L-dopa treatment group. In studies of fetal nigral transplantation,[81–83] although there was a substantial increase in striatal FD uptake post-transplantation, clinical improvement was disappointing. In one subject studied post-mortem after grafting, a marked disparity was noted between DA neuronal counts (that were highly asymmetric) and FD uptake (which was symmetrical).[81] A randomized controlled trial of intraputaminal GDNF infusion in PD similarly failed to demonstrate significant clinical benefit despite increased FD uptake.[87]

This discrepancy between clinical progression and RTI findings could reflect many factors, including potential confounding effects of the dopaminergic medication or other therapy on the surrogate imaging markers rather than on the disease process itself. Levodopa may act to depress DAT binding and AADC activity relative to DA agonists. Also, if DA agonists are indeed neuroprotective or levodopa neurotoxic, this effect might be masked by the higher clinical efficacy of levodopa, especially when using short wash-out periods. Moreover, clinical progression was measured using UPRDS, which reflects a composite of dopaminergic and non-dopaminergic dysfunction in PD;[6] the clinical sign that best reflects the severity of the nigrostriatal lesion is bradykinesia.[9] Clinical rating scales also remain vulnerable to both patient and evaluator subjectivity. In the case of cell-based therapy such as transplantation, grafts may survive, but fail to form synaptic connections with the host striatum. It has indeed been demonstrated that there is lag between improvement in FD uptake following transplant and clinical improvement, which corresponds to improved cerebral blood flow in supplementary motor and prefrontal cortex during performance of a motor task.[88] Thus, assessment of the nigrostriatal DA system alone may be inadequate to assess the overall disease progression in PD.

Further trials are therefore needed in order to determine the contribution of possible confounding factors and to better validate imaging outcomes as biomarkers of disease progression. Proper study design and analysis are required, and the PET data must be interpreted with caution and in the context of the clinical outcome.

Despite the above concerns, functional imaging still offers an objective method of assessing disease progression in PD. In trials of implantation of fetal DA cells[89] into putamen, 18F-dopa PET provided proof of mechanism by detecting increased DA storage capacity after treatment. Also, while not providing a direct readout of dopaminergic function, changes in glucose metabolism can also be used to infer re-establishment of physiological connections. This approach has been successfully applied to the study of glutamic acid decarboxylase (GAD) gene transfer in the STN.[90] Not only does the pattern of glucose metabolism revert to a more physiological profile following treatment, but it can be shown that the change is more in keeping with alteration of STN function as opposed to a simple lesion effect.[91]

Conclusions

It is increasingly recognized that biomarkers are needed to monitor the progression of PD, if new therapies directed at disease modification are to be developed and tested. RTI of striatal dopaminergic function is widely used but is imperfect as a biomarker in PD. However, the association between these measures and clinical change has not always been straightforward.[92] At best, these techniques assess the function of nigrostriatal DA terminals, rather than the number or density of nigral neurons. The potential effects of sprouting, compensatory up- or down-regulation of the receptor/transporter under study, pharmacological influences on receptor/transporter expression, and, indeed, the effects of endogenous DA, are not fully resolved. Some of the most disabling features of advanced PD do not have a major dopaminergic basis and will accordingly not be captured by dopaminergic tracers.[6,93] At this point, while undeniably useful, RTI studies cannot be considered an adequate surrogate endpoint for clinical trials in PD. ■

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