# Molecular Imaging to track Parkinson's disease and atypical parkinsonisms: new imaging frontiers.

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## Abstract

Molecular imaging has proven to be a powerful tool for investigation of parkinsonian disorders. One current challenge is to identify biomarkers of early changes that may predict the clinical trajectory of parkinsonian disorders. Exciting new tracer developments hold the potential for *in vivo* markers of underlying pathology. Herein, we provide an overview of molecular imaging advances, and how these approaches help us to understand Parkinson's Disease and atypical parkinsonisms.

## Introduction

In the last decade, the molecular imaging field has entered a new era of exploration into human brain diseases and has proven to be a powerful tool for investigation of the human brain which is characterized by highly interconnected regions and networks involved in motor, cognitive and behavioral functions. While several of the recent molecular imaging approaches are still under development and probably not yet able to provide definitive answers, they represent valuable

tools to improve our understanding of basic molecular mechanisms and pathophysiological processes underlying parkinsonian disorders. One current challenge is to identify biomarkers of early changes that predict at the group level progression and development of selected manifestations of parkinsonian disorders. On an individual level, it is unclear how helpful molecular imaging techniques (i.e. positron emission tomography [PET] and single-photon emission computed tomography [SPECT]) may help stratify risk for developing motor and behavioral complications. This personalized medicine approach, while still in its infancy in Parkinson's and related disorders, has future potential for identifying subgroups of patients for targeted clinical trials of novel agents. Molecular imaging with newly developed radiopharmaceuticals now has increased potential to reveal underlying pathological processes such as changes in receptors (e.g. dopaminergic and non-dopaminergic), blood flow, metabolism, neuroinflammation, and abnormal protein deposition. Furthermore, some molecular imaging measures may provide biomarkers of target engagement or efficacy for clinical trials. While most imaging studies focused on central nervous system abnormalities, a few interesting studies imaged peripheral organs in PD[1] raising the issue of their practical value. Although most neuroimaging investigations focused on PD, some have addressed the atypical parkinsonisms. This knowledge gap certainly creates the need for new molecular imaging approaches and biomarkers for these atypical parkinsonian disorders still not sufficiently understood. In this article, we will provide an overview of high-affinity radiotracers and molecular imaging advances, and how these approaches have had an impact in understanding PD and atypical parkinsonisms.

## Molecular Imaging of Parkinson's disease and its progression

### *What have we learned from imaging the dopaminergic system ?*

Degeneration of nigrostriatal neurons are responsible for most of the classical motor manifestations of early PD. The underlying pathophysiology in PD includes α-synuclein deposition in cytoplasmic inclusions called Lewy bodies found in residual neurons in areas such as the substantia nigra pars compacta (SNpc), but α-synuclein also may be deposited in dystrophic neurons in striatal or cortical regions (Lewy neurites). Projection neurons like

nigrostriatal afferents have long, poorly myelinated axons and may be particularly vulnerable; Lewy neurites may appear prior to cell body damage.[2]

While dopamine levels (Fig. 1) cannot be measured directly using imaging, several approaches can be used to assess altered function of nigrostriatal dopaminergic nerve terminals (Fig. 2). The most widely accessible approach is the use of a marker for the dopamine transporter (DAT). Several positron- or photon-emitting molecules are available for use with PET or SPECT, respectively. These ligands have varying degrees of selectivity for the DAT over other monoamine reuptake transporters, and pharmacokinetic profiles differ from one tracer to another. This may be of some practical importance because some tracers (e.g. $^{123}$I-β-CIT; $^{123}$I-(–)-2β-Carbomethoxy-3β-(4-iodophenyl)tropane) do not reach steady state for several hours, mandating patients to return for imaging the day following tracer injection, while others ($^{11}$C-d-*threo*-methylphenidate, $^{18}$F-fluoropropyl-β-CIT, $^{123}$I-fluoropropyl-CIT) can be imaged within 1-2 hours of tracer injection. An alternative approach is to label the DAT ligand with $^{99m}$Tc, which is an isotope that is widely used in clinical nuclear medicine. Striatal DAT binding correlates with loss of nigrostriatal dopamine terminals but may only correlate with nigral neurons when that loss does not exceed 50%,[3] whereas direct imaging of midbrain uptake correlates with residual nigral neurons,[4] thus striatal DAT may not reflect disease progression beyond mild-moderate disease.[3,5,6] Striatal or nigral uptake may therefore be a useful imaging biomarker.

A second approach to determining the integrity of the presynaptic dopaminergic terminal is to study binding to the vesicular monoamine transporter type 2 (VMAT2). VMAT2 is responsible for packaging monoamines into their appropriate synaptic vesicles and binding is accordingly not specific to dopamine neurons. However, more than 90% of striatal VMAT2 binding is to dopaminergic nerve terminals. VMAT2 binding is typically studied with $^{11}$C-dihydrotetrabenazine (DTBZ), which is not widely available and studies can therefore only be performed at a few sites. However, an $F^{18}$ labeled radiotracer is now commercially available increasing the accessibility of these measures. VMAT2 binding may be less subject to regulation than DAT binding[7] but this remains controversial.[8] It may have some sensitivity to vesicular dopamine levels, such that VMAT2 binding may be increased in the rare situation where nerve terminals are preserved but are depleted of dopamine.[9]

The original means of studying dopaminergic function was with the use of 6-$^{18}$F-fluoro-L-dopa, which like levodopa, is taken up by monoaminergic neurons, decarboxylated to (fluoro)dopamine and, in the healthy brain, packaged in synaptic vesicles. Also analogous to markers for DAT and VMAT2, fluorodopa uptake declines with disease progression and weakly inversely correlates with clinical severity, at least for mild to moderate degrees of disease severity.[3] However, the interpretation of fluorodopa scans is somewhat more complicated in that uptake reflects not only the (unidirectional) decarboxylation of fluorodopa to fluorodopamine, but also the egress of trapped fluorodopamine from synaptic vesicles. This can be used to estimate dopamine turnover, which increases with disease severity.[10,11]

All of these presynaptic markers of dopaminergic function show a very similar pattern in PD, with asymmetric involvement of the striatum, and a rostral-caudal gradient in which the posterior putamen is maximally affected and the caudate nucleus least. While this rostral-caudal gradient is preserved throughout the course of the illness,[12] the decline in dopaminergic markers over time is better described by an exponential rather than a linear relationship, where the majority of change in the putamen has taken place typically within the first 5 years, akin to post-mortem observations,[12] which have demonstrated almost total loss of tyrosine hydroxylase immunoreactivity within this time.[6]

Concurrent studies of multiple markers reveal that in early disease stages (Hoehn & Yahr Stage I), the threshold for clinical manifestations was 29-44% of normal values for DAT binding, 38-49% for VMAT2 binding and 48-62% for fluorodopa uptake.[13] The more severe involvement of DAT binding may in part reflect downregulation of the DAT in early disease, while lesser involvement of fluorodopa uptake may reflect upregulation of decarboxylase activity in surviving dopamine neurons and/or expression of decarboxylase by serotonergic neurons. In studies conducted in nonhuman primates with unilateral internal carotid infusion of different doses of MPTP, it was found that the loss of only 30-35% of striatal terminals in nonhuman primates were able to generate motor parkinsonism and perhaps, more importantly, motor parkinsonism correlated with the nigral cell counts rather than the terminal field measures.[14] In these animal models, [$^{18}$F]-6-fluorodopa, [$^{11}$C] CFT (DAT biomarker) and $^{11}$C- DTBZ correlated with striatal dopamine and fiber density, a reflection of terminal field function, but interestingly only correlated with nigral cell number within a limited range of cell loss (less than 50%),[3] as

mentioned also above. In contrast, while PET measures of midbrain uptake of either VMAT2 or DAT correlated well throughout the full range of severity of nigrostriatal injury with stereologic counts of nigral dopaminergic neuronal cell bodies,[4] midbrain uptake of fluorodopa did not, thus suggesting the midbrain measures may provide a better biomarker of severity of nigrostriatal injury. Other studies as well showed evidence that serial assessments with multiple markers provide an effective approach to evaluate evolution of dopaminergic depletion in MPTP monkeys.[15,16]

In a cohort of patients with sporadic PD studied longitudinally over several years, de la Fuente-Fernandez et al.[17] estimated that VMAT2 binding declined 17 years prior to disease onset, followed by DAT binding 13 years prior, while decline in fluorodopa uptake occurs last, only 6 years prior to disease onset. However, while fluorodopa uptake does not decline until later, dopamine turnover increases relatively early, an estimated eight years prior to disease onset in sporadic PD,[10] and years or even decades prior to the expected age of onset in patients with *LRRK*2 mutations.[18] Consistent with the latter observation, imaging studies have shown evidence of dopaminergic dysfunction in subjects with a number of pathogenic mutations. The A53T and A53E mutations in the alpha-synuclein gene (*SNCA*) can be associated with early, relatively symmetrical defects in presynaptic dopaminergic function.[19,20] Additionally, recessive Parkin[21-23] and *PINK*1[19] mutations appear to be mostly associated with symmetrical dopaminergic losses, whereas in carriers of more frequent *LRRK2* and *GBA* mutations, the dopaminergic defect is practically indistinguishable from that of sporadic PD.[19,24] In *GBA*-associated PD, the severity of dopaminergic dysfunction is related to the degree of glucocerebrosidase enzymatic activity reduction induced by the specific *GBA* mutation: carriers of mild mutations (e.g. N370S) overlap with PD non-carriers, while PD carriers of severe mutations (e.g. L444P) have a similar phenotype to dementia with Lewy bodies.[25]

Many investigators have proposed that dopamine release may also be a valid method to evaluate dopaminergic functions. This can be estimated by exploiting (Fig. 2) the relatively high affinity of [$^{11}$C]-raclopride, [$^{11}$C]-(+)-PHNO, [$^{18}$F]fallypride or [$^{11}$C]FLB-457 for post-synaptic D2/D3 dopaminergic receptors, such that radioligand binding is subject to competition from endogenous dopamine.[26,27] A change in binding potential can be used to estimate dopamine release at the striatal ([$^{11}$C]-raclopride or [$^{11}$C]-(+)- PHNO) or extrastriatal level ([$^{18}$F]fallypride or [$^{11}$C]FLB-

457 ) in response to medications, behavioral stimuli and brain stimulation techniques. A more detail descriptions of these applications are reported below in the section related to behavioral complications in Parkinson's disease.



***How imaging of non-dopaminergic changes contributed to our current knowledge ?***

A large body of evidence suggests that non–dopaminergic mechanisms may also contribute to the pathophysiology of PD (Fig. 3, 4). Studies conducted with serotonergic PET tracers (Fig. 3, 4) have shed some light on understanding the role of this neurotransmitter. A PET biomarker of the serotonin transporter (SERT), i.e. [$^{11}$C]DASB, has shown a non-linear, gradual loss of presynaptic serotonergic terminal function in the subcortical and cortical areas during PD progression.[28,29] Subsequent reports have suggested its potential role in the pathophysiology of levodopa-induced dyskinesias (LIDs).[30] Using [$^{11}$C]DASB PET together with a series of [$^{11}$C]raclopride PET scans, these studies demonstrated that PD patients with LIDs exhibited relative preserved striatal serotonergic terminals (compared to profound degeneration of dopaminergic terminals), possibly responsible for the synaptic dopaminergic levels, and that oral administration of the 5-HT$_{1A}$ agonist buspirone (prior to levodopa) reduced levodopa-related striatal synaptic dopamine increases and attenuated LIDs.[30,31] Serotonergic PET imaging also indicated similar mechanisms underlying the development of graft-induced dyskinesias (GIDs) in transplanted PD patients. Studies with [$^{11}$C]DASB PET together with dopaminergic biomarkers, i.e. [$^{18}$F]fluorodopa, demonstrated serotonergic hyper-innervation and elevated serotonin/dopamine terminal ratio in the grafted tissue of transplanted PD patients who developed GIDs.[32,33] GIDs were markedly attenuated by systemic administration of 5-HT$_{1A}$ agonist buspirone, which, by reducing transmitter release from serotonergic neurons, may suggest that dyskinesias were likely related to the serotonergic hyper-innervation.

Phosphodiesterase 10A (PDE10A) has a key role in the regulation of dopaminergic signaling in striatal pathways, and in promoting neuronal survival. PDE10A is a basal ganglia expressed dual substrate enzyme, which regulates cAMP and cGMP signaling cascades. PET studies using [$^{11}$C]IMA107, a biomarker of PDE10A *in-vivo*, have demonstrated reduced striatal levels of PDE10A which correlated with PD duration and disease burden scores such as motor disability

and severity of LIDs,[34] thus confirming the complex interaction of dopaminergic and non-dopaminergic mechanisms underlying the pathophysiology of PD.

Consistent with this observation, voxel-based mapping of cerebral blood flow and metabolic activity has also revealed stereotyped, spatially distributed disturbances of regional brain function in PD patients. Functional brain imaging with [$^{18}$F]-fluorodeoxyglucose (FDG) PET has provided a means of detecting and quantifying highly specific spatial covariance patterns associated with a variety of neurodegenerative disorders including PD.[35] The PD-related metabolic pattern (PDRP), identified in resting state metabolic imaging data analyzed using spatial covariance mapping, is characterized by increased pallidothalamic and pontine metabolic activity, associated with reductions in premotor cortex and parietal association areas. Expression values for the PDRP measured in individual subjects correlates significantly with loss of presynaptic nigrostriatal dopaminergic integrity, as well as with independent clinical ratings of motor dysfunction.[36,37] Notably, topographically similar metabolic network abnormalities have been recently identified in nonhuman primates with experimental parkinsonism due to systemic MPTP exposure.[38,39] In a recent blinded surgical trial of gene therapy for PD, the rate of PDRP progression measured over one year was not affected by placebo treatment.[40] In contrast, reductions in PDRP expression have been found consistently during levodopa therapy and STN-DBS and significantly correlated with clinical improvement.[41] This suggests that PDRP can be considered a reliable biomarker of treatment response. PDRP expression is also sensitive to network changes occurring prior to the appearance of motor symptoms. Expression levels of this network have recently been found to be abnormally elevated in the clinically unaffected hemisphere of early PD patients and in "preclinical" subjects with REM sleep behavior disorder.[36,42]

Neuroinflammation is also considered to play an important role in PD. [43, 44, 45] Translocator protein 18 kDa (TSPO) has been investigated as a potential biomarker of inflammation. Elevated TSPO expression was primarily quantified using [$^{11}$C]*(R)*PK11195 PET. To date, a few studies have investigated neuroinflammation in PD patients using [$^{11}$C]*(R)*PK11195 PET. While some studies have found elevated TSPO binding in the nigro-striatal regions,[46-48] others did not support these observations.[49] These limitations have prompted the development of second-generation TSPO radioligands (i.e. [$^{11}$C]PBR28; [$^{18}$F]-FEPPA, etc.) which present three patterns of binding

affinity based on a genetic polymorphism: low-affinity binders (LABs), mixed-affinity binders (MABs) and high-affinity binders (HABs).[50] These different genotype binding affinity patterns account for some of the large inter-individual variability in the outcome measures[50] and can be predicted by a single-nucleotide polymorphism (SNP), *rs*6971 located in the exon 4 of the *TSPO* gene resulting in a nonconservative amino-acid substitution at position 147 from alanine to threonine (Ala147Thr) in the fifth transmembrane domain of the TSPO protein. Using these second-generation TSPO radioligands (i.e. [$^{18}$F]-FEPPA), Koshimori et al.,[43] while noting a significant genotype effect (MABs vs HABs) on the [$^{18}$F]-FEPPA volume distribution (Vt), did not observe any disease effect on differential TSPO binding in the striatum of PD patients. Other studies however in individuals with Alzheimer's disease have shown evidence of significant increase in [$^{18}$F]-FEPPA Vt in HABs but not MABs.[51]

## Mild Cognitive Impairment and Dementia in Parkinson's disease

***Have different imaging biomarkers helped to understand cognitive deterioration ?***

The etiology of cognitive decline in PD is heterogeneous. Imaging biomarker studies of cognitive impairment in PD have targeted neurotransmitter systems, pathological protein deposits, and glucose metabolic or perfusion changes. Prospective evaluation of glucose metabolic changes have shown that incident dementia initially may present as a predominant hypometabolic posterior cortical pathology involving the visual association cortex, inferior parietal and temporal regions, the posterior cingulum and precuneus in PD.[52,53] Subsequent progression to dementia is associated with mixed subcortical, including the thalamus and caudate nuclei, and widespread cortical changes that involve the anterior cortices as well.[52,54] Spatial covariance mapping has been particularly useful in providing information regarding the network topography that underlies cognitive dysfunction in PD.[35] Indeed, this approach has revealed a distinct PD cognition-related pattern (PDCP) characterized by metabolic reductions in the medial prefrontal, premotor, and parietal association regions, with relative increases in the cerebellar vermis and dentate nuclei. In cross-sectional analyses, increased PDCP expression is associated with more severe cognitive impairment. PDCP expression is abnormally elevated even in PD patients without evidence of cognitive impairment, and highest in those with dementia.[35] Of note,

PD subjects whose executive function improved with levodopa administration ("responders") also exhibited concurrent reductions in PDCP expression. By contrast, PD subjects showing no cognitive improvement ("non-responders") exhibited no change in PDCP expression with levodopa.[55] Both groups, however, exhibited significant levodopa-mediated reductions in PDRP expression, underscoring the functional distinction between the cognitive and motor networks. These findings together with a recent study on glucose metabolism in groups of patients along the spectrum of parkinsonism to dementia (PD, PDD, DLB, AD) support the notion that glucose metabolism patterns may reflect more the clinical syndrome than the underlying pathology.[56]

An intriguing dual syndrome cognitive hypothesis has been proposed, which posits that the high frequency of fronto-striatal executive dysfunction in PD may relate to common dopaminergic deficits[57,58] and that the development of dementia is associated with more widespread and posterior cortical changes secondary to additional pathologies, including cholinergic deficits.[59,60] Although dopaminergic denervation affects specific cognitive functions in PD,[58] striatal and limbo-frontal dopaminergic changes are present in non-demented PD subjects,[58] but their presence is not sufficient to explain the full development of dementia in PD.[61] In contrast, greater cholinergic denervation is shown consistently in PD dementia compared to PD.[61,62] These observations support a more complex pathophysiological model of interacting dopaminergic (Fig. 1) and cholinergic degenerative (Fig. 3, 4) changes producing cognitive dysfunctions in PD.[63-65] However, imaging studies confirmed as well a significant relationship between *in vivo* measures of elevated cortical and, in particular, striatal β- amyloid deposits (measured with [$^{11}$C]PIB) and greater cognitive impairment in PD.[66,67] However, the risk of having an abnormal [$^{11}$C] PIB PET study in PD with dementia substantially underestimated the risk of abnormal β-deposition in the brain at autopsy in people with PD and dementia.[68,69] Interestingly, β-amyloid deposition measured either in vivo with [$^{11}$C] PIB or at autopsy with immunohistochemistry did not necessarily reflect co-existing AD, since those with dementia due to AD would present marked pathologic deposition of both β-amyloid and tau. Furthermore, the distribution of β-amyloid measured with [$^{11}$C] PIB in PDD has a significantly different pattern in the brain demonstrated by principal components analysis compared to those with AD.[70] Together these data indicate the relevance of β-amyloid brain deposition in PD but suggests that this does not merely reflect the full spectrum of AD pathology.

All of these data demonstrate that neurotransmitter and proteinopathy changes have independent and incremental contributions to the cognitive syndrome in PD.[65] However, other factors (i.e. neuroinflammation) may also play a role. In fact, it has been shown that there may exist a direct relationship between β-amyloid load and levels of microglial activation in PD dementia subjects,[71] suggesting that neuroinflammation may be an early phenomenon, before the dementia onset, and that amyloid along with microglial activation could together contribute not only to the local neuronal dysfunction, but also to the more remote neuronal disconnection.[71] Thus for future studies, there is a need for new ligands for not only neurotransmission, in particular norepinephrine, but also new neuroinflammatory (besides TSPO binding tracers) and proteinopathy targets, especially tracers to visualize neurofibrillary tau and α-synuclein protein aggregates for more comprehensive understanding of the cognitive impairment syndrome in PD.

## Behavioral and Affective Complications in Parkinson's disease

***What molecular imaging has taught us about behavioral spectrum disorders***

There is evidence of a behavioral spectrum disorder ranging from hypo-dopaminergic levels responsible for apathy, anxiety, and depression as described in the withdrawal dopaminergic syndrome to hyper-dopaminergic syndrome including impulse control disorders (ICDs), hallucinations and psychosis.[72,73] Often depression may manifest before the diagnosis of PD, however there is considerable evidence suggesting that this complication can be associated with a more widespread neurodegenerative process. Imaging reports seem to suggest involvement of both dopaminergic and serotonergic systems. While studies using the dopamine transporter radioligand (i.e. TRODAT-1) found significantly higher DAT density in the striatum of depressed PD patients,[74] other investigations with serotonin transporter (i.e. [$^{11}$C]DASB) showed abnormal serotonergic neurotransmission in the raphe nuclei and limbic structures which correlated with depression measures.[75] The serotonergic alteration in depression was confirmed by another PET study using $^{18}$F-MPPF, a selective serotonin 1A receptor antagonist.[76] Apathy may also occur in up to 40% of PD patients and, although clinically distinct from depression, the two are often comorbid.[72] Anatomical and imaging reports have provided evidence that network abnormalities within the prefrontal–striatal circuit can lead to an apathetic behavior. Classically,

apathy is the result of a disruption of 'emotional-affective' mechanisms linked to the ventral striatum, ventromedial prefrontal cortex (PFC) and amygdala.[77] PET studies with D2/D3 receptor antagonist, [11C]-raclopride, have shown several differences in dopaminergic binding and transmission in the mesocorticolimbic system between apathetic and non-apathetic PD patients.[78] [11C]-raclopride binding potential was increased in apathetic PD patients in the orbitofrontal cortex (OFC), cingulate cortex, dorsolateral prefrontal cortex (DLPFC), amygdala as well as in the striatum, implying either (reactive) increase in D2/D3 receptor expression and/or reduction in endogenous synaptic dopamine. Other PET studies with [$^{11}$C]RTI-32, a ligand with affinity to both dopamine and noradrenaline transporters, confirmed that the degree of apathy severity was inversely correlated with [$^{11}$C]RTI-32 binding in the ventral striatum.[79] Taken together, these observations seem to suggest that apathy in PD may result from severe dopamine abnormalities in the mesocorticolimbic system, leading to an impaired emotion reactivity and poor decision-making processes,[77] as also demonstrated in nonhuman primate studies of apathetic behaviors after MPTP.[80,81] However, from more recent evidence, the mechanism of apathy, depression and anxiety in PD may be more complex and may differ according to the stage of the disease. In fact, in de novo PD, serotonergic rather than dopaminergic degeneration appears to play a significant role in this non-motor triad.[82]

While certain behavioral complications are often inherent to the disease process, others are mainly associated with symptomatic treatments. Dopamine agonists for example have been implicated in the development of impulse control disorders (ICDs).[83] Susceptibility to these behavioral addictions is associated with increased striatal dopamine release[84-86] and reduced DAT binding in the ventral striatum.[87] While abnormalities in dopaminergic processing in the ventral striatum are critical for the development of ICDs, prefrontal mechanisms may also play an important inhibitory role in these behaviors. Activation PET studies with $H_2[^{15}O]$ before and after administration of a dopamine agonist in PD with and without gambling behavior found changes in brain areas implicated in impulse control and response inhibition (lateral orbitofrontal cortex, rostral cingulate zone, amygdala).[88] Although the agonist significantly increased regional cerebral blood flow (rCBF) in these areas in healthy subjects, gamblers showed, in contrast, a significant reduction of activity. A subsequent PET study using the extrastriatal dopamine receptor ligand [$^{11}$C]FLB-457[89] found significant abnormalities in D2 receptor binding in the

OFC and anterior cingulate cortex (ACC) in PD patients with PG, thus confirming the role of prefrontal control in the development of ICDs. Similarly, [$^{18}$F]fluorodopa PET has shown abnormalities in the OFC of PD patients with ICDs.[90] In PD patients, deep brain stimulation of the subthalamic nucleus (STN-DBS) may also contribute to certain impulsive behavior associated with high-conflict decisions.[91,92] A regional cerebral blood flow study with $H_2$[$^{15}$O] PET during a Go/NoGo task showed a relationship between motor improvement and response inhibition. In particular, STN-DBS affected response inhibition, as revealed by an increase in commission errors in NoGo trials[91] and stop signal task.[92] These behavioral changes were accompanied by changes in synaptic activity characterized by a reduced activation in the cortical networks associated with proactive and reactive response inhibition. These observations suggest that modulation of STN with DBS, although it improves motor functions, may tend in parallel to favor the appearance of certain impulsive behaviors by acting on mechanisms involved in response initiation and/or selection.[91] However, to date, the impact of DBS (and its interaction with dopamine agonist reduction) on the development of ICD is unclear and still quite controversial, as studies using different approaches have shown conflicting results.[93-95]

Fatigue is a common non-motor symptom in PD. Recent studies have reported that PD patients with higher level of fatigue may show anti-correlated metabolic changes in cortical regions associated with the salience (i.e., right insular region) and default (i.e., bilateral posterior cingulate cortex) networks.[96] Other studies of dopaminergic and serotoninergic function in PD patients with and without fatigue demonstrated a serotoninergic denervation in the basal ganglia and related limbic circuits.[97] PD patients with fatigue had significantly lower SERT binding than patients without fatigue in the basal ganglia structure.[97] Additionally, voxel-based analysis identified reduced dopaminergic activity in caudate and insula, and further SERT reductions in cingulate and amygdala in the fatigue group. All together these findings provide the rationale for treatment strategies aiming to increase brain level of serotonin and serotoninergic transmission as potential treatment of this common complication in PD patients.

## Differentiating atypical Parkinsonisms from PD

***Is molecular imaging helping us?***

To date most molecular imaging studies in parkinsonism have focused on investigating either dopaminergic changes or cerebral blood flow and metabolism. Progressive Supranuclear Palsy (PSP) and Corticobasal Syndrome (CBS) are common forms of atypical parkinsonism (APS), and in early stages, can be sometimes quite difficult to diagnose as they can overlap clinically with PD, and other parkinsonian syndromes including multiple system atrophy (MSA). Previous imaging studies have reported in CBS either asymmetric hypoperfusion or reduced metabolism mainly in the striatum as well as parietal and frontal cortex contralateral to the affected limb.[98] CBS may also be variably associated with asymmetric striatal dopamine denervation (e.g. [99]). Similarly, PSP patients may present with a variable pattern of hypometabolism in the fronto-striatal-thalamic regions depending on the clinical presentation and progression.[98,100] Families with mutations in the progranulin gene presented profound imaging changes of asymmetric hypoperfusion on SPECT and parietal atrophy.[101,102] Subsequent work in CBS identified that hypoperfusion within the left inferior parietal lobule, including the left angular gyrus was associated with more severe ideomotor apraxia. Voxel-based spatial covariance mapping has also been used to identify disease-specific networks for MSA, PSP, and CBD.[103,104] Importantly, an automated logistic regression algorithm based on pattern expression values has been developed to aid in discriminating individuals with idiopathic PD from those with atypical parkinsonian syndromes, and in differentiating among the various forms of APS. This approach had excellent diagnostic specificity in an original data set[105] and in a subsequent validation sample.[106] Other methods such as relevance vector machine analysis have also been used for single case classification with promising results.[107,108] Prospective validation studies are needed before the relative utility of these methods can be determined. Neuroinflammation may play an important role in various atypical parkinsonisms. To date, only a few studies have investigated neuroinflammation in these disorders using mainly first generation of radiotracers, i.e. [$^{11}$C]*(R)*PK11195.[109] While the findings are highly suggestive, they require further confirmation.

Other studies assessed the diagnostic value of dopaminergic tracers using ($^{18}$F)-FP-CIT PET in differentiating PSP and MSA from PD.[110] Compared to PD, PSP and MSA have more prominent dopamine transporter loss in the anterior caudate and ventral putamen, respectively. However, it should be emphasized that the pattern of presynaptic dopaminergic impairment is not felt to

reliably differentiate among various neurodegenerative forms of parkinsonism. Studies in which presynaptic dopamine markers are combined with measures of postsynaptic dopamine receptors may help differentiate PD from atypical parkinsonian syndromes, but not between these various syndromes. Except in a few highly specialized centers, neuroimaging has had a very limited diagnostic value in the differential diagnosis of PSP, CBS and other tauopathies. For this reason, the development of PET radiotracers specific for tau represents one of the most active and challenging areas in molecular imaging.

Several groups have recently reported encouraging results toward the development of selective tau imaging agents.[111,112] [$^{18}$F]T807 (also known as [$^{18}$F]-AV-1451) has been reported having excellent selectivity for paired helical filaments of tau.[111,112] This tracer demonstrated high-affinity and selectivity as well as favorable in-vivo properties, making this a potentially promising candidate as an imaging agent for tau.[111] Another tracer, [$^{11}$C]PBB3, has also been recently applied to human studies, providing PET demonstration of spreading tau pathologies in transition from normal aging to advanced Alzheimer's disease.[113] PET imaging of sporadic 4-repeat tau pathologies in PSP and CBD is currently being conducted, and preliminary data have indicated an increased retention of [$^{11}$C]PBB3 in multiple brain areas, including white matter, in patients with these disorders relative to age-matched controls. Studies of in-vitro binding assays using brain homogenates found that the two tau probes, PBB3 and T807, do not compete with each other for binding sites in PSP tau aggregates. Other investigations with [$^{18}$F]-AV-1451 (i.e. [$^{18}$F]T807) in PSP and CBS have shown preliminary findings with either increase[114] or no retention.[115]

## Future directions of molecular imaging: need for harmonization and multi-center collaboration

Exciting new tracer developments hold the potential for *in vivo* markers of underlying pathology, which is of particular interest for interventions directly targeting protein aggregation. However, with increasingly diverse and sophisticated imaging approaches, it is now becoming more problematic than ever for non-experts to assess the validity and significance of new studies. The scientific community should therefore move toward common standards and harmonization in molecular imaging. Good scientific practice criteria including sample size, correction for

multiple comparisons, correction for the effects of age, motion, and partial volume effects are among the most important issues to address. Anticipating excessive use of the term "biomarker" in imaging studies, we propose to use standardized terminology, which may help in the design future experiments, especially when looking for surrogate markers in interventional studies. In line with the FDA/NIH BEST Resource (2016) propositions, a biomarker is a defined as a characteristic that is measured as an indicator of a biological (pathogenic) process. A diagnostic biomarker should increase diagnostic accuracy for pathological or clinical entities in comparison to clinical judgment alone. In agreement with this definition, certain imaging characteristics may also serve to enrich specific features in a trial population (for example as a target verification tool in case of tau PET). Monitoring biomarkers are measured serially and used to detect a change in the degree or extent of disease. This kind of imaging characteristic may serve as a biomarker that may predict clinical efficacy but not likely to act as a surrogate endpoint since such an imaging biomarker would not reflect unintended side effects. For PD, most valuable biomarkers would certainly be prodromal diagnostic biomarkers and monitoring biomarkers for disease progression at early/prodromal stages. For atypical parkinsonism, there often is a mismatch between clinical and pathological entities (e.g. CBS with AD pathology, PSP pathology with different clinical phenotypes).[116,117] Therefore, diagnostic biomarkers in atypical parkinsonism should not be regarded as diagnostic for a clinical entity, unless the biomarker is pathologically validated. While certainly fruitful, this kind of endeavor only seems feasible in a large multi-center studies strategically focusing on the integration of postmortem information.

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## Authors' Roles

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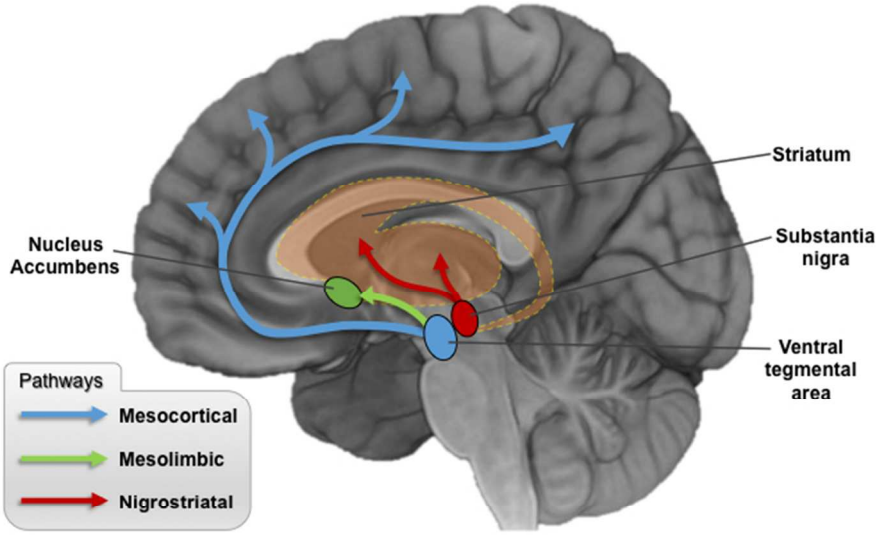
## Figure Legends

**Figure 1** depicts the ascending nigrostriatal (red), mesolimbic (green), and mesocortical (blue) dopaminergic pathways.

**Figure 2** shows dopaminergic nerve terminal and various PET radiotracers for the assessment of its integrity.

**Figure 3** (top) depicts the distribution of the serotonergic pathways (Yellow), (bottom) depicts the distribution of the cholinergic pathway (Red).

**Figure 4** shows cholinergic (left) and serotonergic (right) nerve terminal and various PET radiotracers for the assessment of their integrity.

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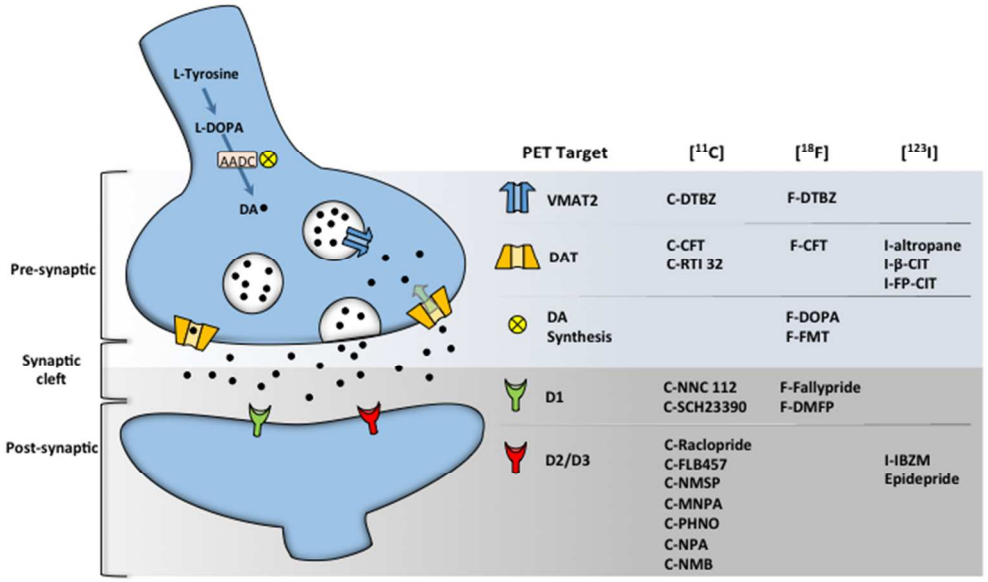


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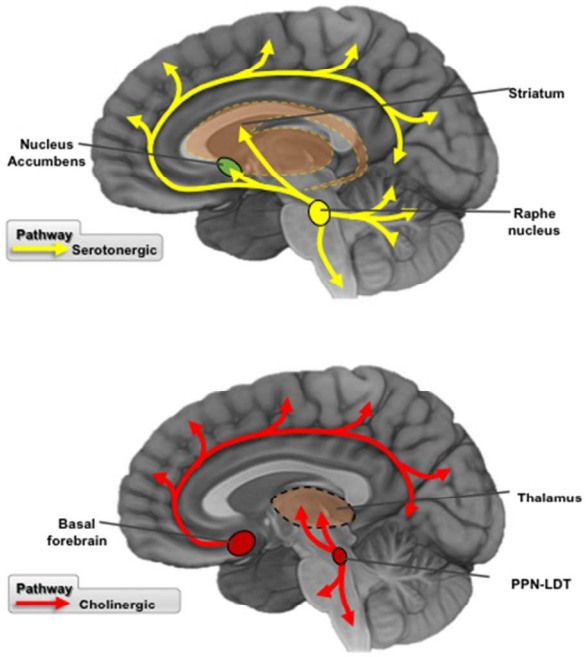


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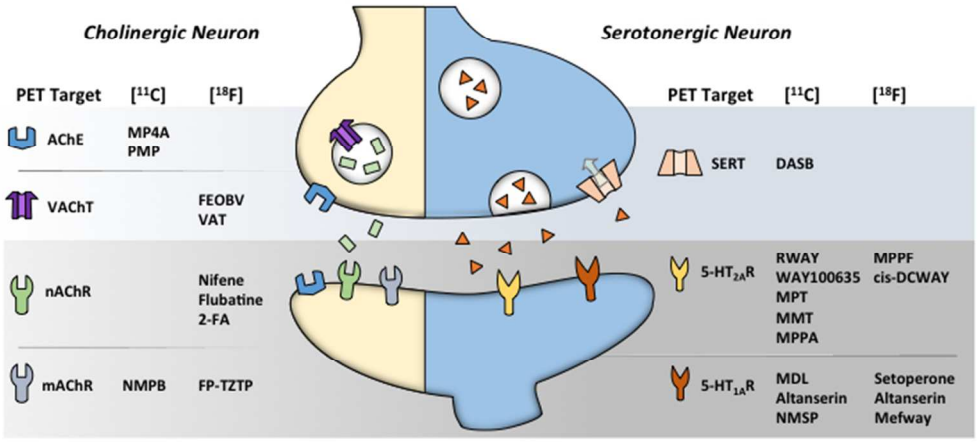


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# Molecular Imaging to track Parkinson's disease and atypical parkinsonisms: new imaging frontiers.

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## Abstract

Molecular imaging has proven to be a powerful tool for investigation of parkinsonian disorders. One current challenge is to identify biomarkers of early changes that may predict the clinical trajectory of parkinsonian disorders. Exciting new tracer developments hold the potential for *in vivo* markers of underlying pathology. Herein, we provide an overview of molecular imaging advances, and how these approaches help us to understand Parkinson's Disease and atypical parkinsonisms.

## Introduction

In the last decade, the molecular imaging field has entered a new era of exploration into human brain diseases and has proven to be a powerful tool for investigation of the human brain which is characterized by highly interconnected regions and networks involved in motor, cognitive and behavioral functions. While several of the recent molecular imaging approaches are still under development and probably not yet able to provide definitive answers, they represent valuable

tools to improve our understanding of basic molecular mechanisms and pathophysiological processes underlying parkinsonian disorders. One current challenge is to identify biomarkers of early changes that predict at the group level progression and development of selected manifestations of parkinsonian disorders. On an individual level, it is unclear how helpful molecular imaging techniques (i.e. positron emission tomography [PET] and single-photon emission computed tomography [SPECT]) may help stratify risk for developing motor and behavioral complications. This personalized medicine approach, while still in its infancy in Parkinson's and related disorders, has future potential for identifying subgroups of patients for targeted clinical trials of novel agents. Molecular imaging with newly developed radiopharmaceuticals now has increased potential to reveal underlying pathological processes such as changes in receptors (e.g. dopaminergic and non-dopaminergic), blood flow, metabolism, neuroinflammation, and abnormal protein deposition. Furthermore, some molecular imaging measures may provide biomarkers of target engagement or efficacy for clinical trials. While most imaging studies focused on central nervous system abnormalities, a few interesting studies imaged peripheral organs in PD[1] raising the issue of their practical value. Although most neuroimaging investigations focused on PD, some have addressed the atypical parkinsonisms. This knowledge gap certainly creates the need for new molecular imaging approaches and biomarkers for these atypical parkinsonian disorders still not sufficiently understood. In this article, we will provide an overview of high-affinity radiotracers and molecular imaging advances, and how these approaches have had an impact in understanding PD and atypical parkinsonisms.

## Molecular Imaging of Parkinson's disease and its progression

### *What have we learned from imaging the dopaminergic system ?*

Degeneration of nigrostriatal neurons are responsible for most of the classical motor manifestations of early PD. The underlying pathophysiology in PD includes α-synuclein deposition in cytoplasmic inclusions called Lewy bodies found in residual neurons in areas such as the substantia nigra pars compacta (SNpc), but α-synuclein also may be deposited in dystrophic neurons in striatal or cortical regions (Lewy neurites). Projection neurons like

nigrostriatal afferents have long, poorly myelinated axons and may be particularly vulnerable; Lewy neurites may appear prior to cell body damage.[2]

While dopamine levels (Fig. 1) cannot be measured directly using imaging, several approaches can be used to assess altered function of nigrostriatal dopaminergic nerve terminals (Fig. 2). The most widely accessible approach is the use of a marker for the dopamine transporter (DAT). Several positron- or photon-emitting molecules are available for use with PET or SPECT, respectively. These ligands have varying degrees of selectivity for the DAT over other monoamine reuptake transporters, and pharmacokinetic profiles differ from one tracer to another. This may be of some practical importance because some tracers (e.g. $^{123}$I-β-CIT; $^{123}$I-(–)-2β-Carbomethoxy-3β-(4-iodophenyl)tropane) do not reach steady state for several hours, mandating patients to return for imaging the day following tracer injection, while others ($^{11}$C-d-*threo*-methylphenidate, $^{18}$F-fluoropropyl-β-CIT, $^{123}$I-fluoropropyl-CIT) can be imaged within 1-2 hours of tracer injection. An alternative approach is to label the DAT ligand with $^{99m}$Tc, which is an isotope that is widely used in clinical nuclear medicine. Striatal DAT binding correlates with loss of nigrostriatal dopamine terminals but may only correlate with nigral neurons when that loss does not exceed 50%,[3] whereas direct imaging of midbrain uptake correlates with residual nigral neurons,[4] thus striatal DAT may not reflect disease progression beyond mild-moderate disease.[3,5,6] Striatal or nigral uptake may therefore be a useful imaging biomarker.

A second approach to determining the integrity of the presynaptic dopaminergic terminal is to study binding to the vesicular monoamine transporter type 2 (VMAT2). VMAT2 is responsible for packaging monoamines into their appropriate synaptic vesicles and binding is accordingly not specific to dopamine neurons. However, more than 90% of striatal VMAT2 binding is to dopaminergic nerve terminals. VMAT2 binding is typically studied with $^{11}$C-dihydrotetrabenazine (DTBZ), which is not widely available and studies can therefore only be performed at a few sites. However, an $F^{18}$ labeled radiotracer is now commercially available increasing the accessibility of these measures. VMAT2 binding may be less subject to regulation than DAT binding[7] but this remains controversial.[8] It may have some sensitivity to vesicular dopamine levels, such that VMAT2 binding may be increased in the rare situation where nerve terminals are preserved but are depleted of dopamine.[9]

The original means of studying dopaminergic function was with the use of 6-$^{18}$F-fluoro-L-dopa, which like levodopa, is taken up by monoaminergic neurons, decarboxylated to (fluoro)dopamine and, in the healthy brain, packaged in synaptic vesicles. Also analogous to markers for DAT and VMAT2, fluorodopa uptake declines with disease progression and weakly inversely correlates with clinical severity, at least for mild to moderate degrees of disease severity.[3] However, the interpretation of fluorodopa scans is somewhat more complicated in that uptake reflects not only the (unidirectional) decarboxylation of fluorodopa to fluorodopamine, but also the egress of trapped fluorodopamine from synaptic vesicles. This can be used to estimate dopamine turnover, which increases with disease severity.[10,11]

All of these presynaptic markers of dopaminergic function show a very similar pattern in PD, with asymmetric involvement of the striatum, and a rostral-caudal gradient in which the posterior putamen is maximally affected and the caudate nucleus least. While this rostral-caudal gradient is preserved throughout the course of the illness,[12] the decline in dopaminergic markers over time is better described by an exponential rather than a linear relationship, where the majority of change in the putamen has taken place typically within the first 5 years, akin to post-mortem observations,[12] which have demonstrated almost total loss of tyrosine hydroxylase immunoreactivity within this time.[6]

Concurrent studies of multiple markers reveal that in early disease stages (Hoehn & Yahr Stage I), the threshold for clinical manifestations was 29-44% of normal values for DAT binding, 38-49% for VMAT2 binding and 48-62% for fluorodopa uptake.[13] The more severe involvement of DAT binding may in part reflect downregulation of the DAT in early disease, while lesser involvement of fluorodopa uptake may reflect upregulation of decarboxylase activity in surviving dopamine neurons and/or expression of decarboxylase by serotonergic neurons. In studies conducted in nonhuman primates with unilateral internal carotid infusion of different doses of MPTP, it was found that the loss of only 30-35% of striatal terminals in nonhuman primates were able to generate motor parkinsonism and perhaps, more importantly, motor parkinsonism correlated with the nigral cell counts rather than the terminal field measures.[14] In these animal models, [$^{18}$F]-6-fluorodopa, [$^{11}$C] CFT (DAT biomarker) and $^{11}$C- DTBZ correlated with striatal dopamine and fiber density, a reflection of terminal field function, but interestingly only correlated with nigral cell number within a limited range of cell loss (less than 50%),[3] as

mentioned also above. In contrast, while PET measures of midbrain uptake of either VMAT2 or DAT correlated well throughout the full range of severity of nigrostriatal injury with stereologic counts of nigral dopaminergic neuronal cell bodies,[4] midbrain uptake of fluorodopa did not, thus suggesting the midbrain measures may provide a better biomarker of severity of nigrostriatal injury. Other studies as well showed evidence that serial assessments with multiple markers provide an effective approach to evaluate evolution of dopaminergic depletion in MPTP monkeys.[15,16]

In a cohort of patients with sporadic PD studied longitudinally over several years, de la Fuente-Fernandez et al.[17] estimated that VMAT2 binding declined 17 years prior to disease onset, followed by DAT binding 13 years prior, while decline in fluorodopa uptake occurs last, only 6 years prior to disease onset. However, while fluorodopa uptake does not decline until later, dopamine turnover increases relatively early, an estimated eight years prior to disease onset in sporadic PD,[10] and years or even decades prior to the expected age of onset in patients with *LRRK2* mutations.[18] Consistent with the latter observation, imaging studies have shown evidence of dopaminergic dysfunction in subjects with a number of pathogenic mutations. The A53T and A53E mutations in the alpha-synuclein gene (*SNCA*) can be associated with early, relatively symmetrical defects in presynaptic dopaminergic function.[19,20] Additionally, recessive Parkin[21-23] and *PINK*1[19] mutations appear to be mostly associated with symmetrical dopaminergic losses, whereas in carriers of more frequent *LRRK2* and *GBA* mutations, the dopaminergic defect is practically indistinguishable from that of sporadic PD.[19,24] In *GBA*-associated PD, the severity of dopaminergic dysfunction is related to the degree of glucocerebrosidase enzymatic activity reduction induced by the specific *GBA* mutation: carriers of mild mutations (e.g. N370S) overlap with PD non-carriers, while PD carriers of severe mutations (e.g. L444P) have a similar phenotype to dementia with Lewy bodies.[25]

Many investigators have proposed that dopamine release may also be a valid method to evaluate dopaminergic functions. This can be estimated by exploiting (Fig. 2) the relatively high affinity of [$^{11}$C]-raclopride, [$^{11}$C]-(+)-PHNO, [$^{18}$F]fallypride or [$^{11}$C]FLB-457 for post-synaptic D2/D3 dopaminergic receptors, such that radioligand binding is subject to competition from endogenous dopamine.[26,27] A change in binding potential can be used to estimate dopamine release at the striatal ([$^{11}$C]-raclopride or [$^{11}$C]-(+)- PHNO) or extrastriatal level ([$^{18}$F]fallypride or [$^{11}$C]FLB-

457 ) in response to medications, behavioral stimuli and brain stimulation techniques. A more detail descriptions of these applications are reported below in the section related to behavioral complications in Parkinson's disease.

***How imaging of non-dopaminergic changes contributed to our current knowledge ?***

A large body of evidence suggests that non–dopaminergic mechanisms may also contribute to the pathophysiology of PD (Fig. 3, 4). Studies conducted with serotonergic PET tracers (Fig. 3, 4) have shed some light on understanding the role of this neurotransmitter. A PET biomarker of the serotonin transporter (SERT), i.e. [$^{11}$C]DASB, has shown a non-linear, gradual loss of presynaptic serotonergic terminal function in the subcortical and cortical areas during PD progression.[28,29] Subsequent reports have suggested its potential role in the pathophysiology of levodopa-induced dyskinesias (LIDs).[30] Using [$^{11}$C]DASB PET together with a series of [$^{11}$C]raclopride PET scans, these studies demonstrated that PD patients with LIDs exhibited relative preserved striatal serotonergic terminals (compared to profound degeneration of dopaminergic terminals), possibly responsible for the synaptic dopaminergic levels, and that oral administration of the 5-HT$_{1A}$ agonist buspirone (prior to levodopa) reduced levodopa-related striatal synaptic dopamine increases and attenuated LIDs.[30,31] Serotonergic PET imaging also indicated similar mechanisms underlying the development of graft-induced dyskinesias (GIDs) in transplanted PD patients. Studies with [$^{11}$C]DASB PET together with dopaminergic biomarkers, i.e. [$^{18}$F]fluorodopa, demonstrated serotonergic hyper-innervation and elevated serotonin/dopamine terminal ratio in the grafted tissue of transplanted PD patients who developed GIDs.[32,33] GIDs were markedly attenuated by systemic administration of 5-HT$_{1A}$ agonist buspirone, which, by reducing transmitter release from serotonergic neurons, may suggest that dyskinesias were likely related to the serotonergic hyper-innervation.

Phosphodiesterase 10A (PDE10A) has a key role in the regulation of dopaminergic signaling in striatal pathways, and in promoting neuronal survival. PDE10A is a basal ganglia expressed dual substrate enzyme, which regulates cAMP and cGMP signaling cascades. PET studies using [$^{11}$C]IMA107, a biomarker of PDE10A *in-vivo*, have demonstrated reduced striatal levels of PDE10A which correlated with PD duration and disease burden scores such as motor disability

and severity of LIDs,[34] thus confirming the complex interaction of dopaminergic and non-dopaminergic mechanisms underlying the pathophysiology of PD.

Consistent with this observation, voxel-based mapping of cerebral blood flow and metabolic activity has also revealed stereotyped, spatially distributed disturbances of regional brain function in PD patients. Functional brain imaging with [$^{18}$F]-fluorodeoxyglucose (FDG) PET has provided a means of detecting and quantifying highly specific spatial covariance patterns associated with a variety of neurodegenerative disorders including PD.[35] The PD-related metabolic pattern (PDRP), identified in resting state metabolic imaging data analyzed using spatial covariance mapping, is characterized by increased pallidothalamic and pontine metabolic activity, associated with reductions in premotor cortex and parietal association areas. Expression values for the PDRP measured in individual subjects correlates significantly with loss of presynaptic nigrostriatal dopaminergic integrity, as well as with independent clinical ratings of motor dysfunction.[36,37] Notably, topographically similar metabolic network abnormalities have been recently identified in nonhuman primates with experimental parkinsonism due to systemic MPTP exposure.[38,39] In a recent blinded surgical trial of gene therapy for PD, the rate of PDRP progression measured over one year was not affected by placebo treatment.[40] In contrast, reductions in PDRP expression have been found consistently during levodopa therapy and STN-DBS and significantly correlated with clinical improvement.[41] This suggests that PDRP can be considered a reliable biomarker of treatment response. PDRP expression is also sensitive to network changes occurring prior to the appearance of motor symptoms. Expression levels of this network have recently been found to be abnormally elevated in the clinically unaffected hemisphere of early PD patients and in "preclinical" subjects with REM sleep behavior disorder.[36,42]

Neuroinflammation is also considered to play an important role in PD. [43, 44, 45] Translocator protein 18 kDa (TSPO) has been investigated as a potential biomarker of inflammation. Elevated TSPO expression was primarily quantified using [$^{11}$C]*(R)*PK11195 PET. To date, a few studies have investigated neuroinflammation in PD patients using [$^{11}$C]*(R)*PK11195 PET. While some studies have found elevated TSPO binding in the nigro-striatal regions,[46-48] others did not support these observations.[49] These limitations have prompted the development of second-generation TSPO radioligands (i.e. [$^{11}$C]PBR28; [$^{18}$F]-FEPPA, etc.) which present three patterns of binding

affinity based on a genetic polymorphism: low-affinity binders (LABs), mixed-affinity binders (MABs) and high-affinity binders (HABs).[50] These different genotype binding affinity patterns account for some of the large inter-individual variability in the outcome measures[50] and can be predicted by a single-nucleotide polymorphism (SNP), *rs*6971 located in the exon 4 of the *TSPO* gene resulting in a nonconservative amino-acid substitution at position 147 from alanine to threonine (Ala147Thr) in the fifth transmembrane domain of the TSPO protein. Using these second-generation TSPO radioligands (i.e. [$^{18}$F]-FEPPA), Koshimori et al.,[43] while noting a significant genotype effect (MABs vs HABs) on the [$^{18}$F]-FEPPA volume distribution (Vt), did not observe any disease effect on differential TSPO binding in the striatum of PD patients. Other studies however in individuals with Alzheimer's disease have shown evidence of significant increase in [$^{18}$F]-FEPPA Vt in HABs but not MABs.[51]

## Mild Cognitive Impairment and Dementia in Parkinson's disease

***Have different imaging biomarkers helped to understand cognitive deterioration ?***

The etiology of cognitive decline in PD is heterogeneous. Imaging biomarker studies of cognitive impairment in PD have targeted neurotransmitter systems, pathological protein deposits, and glucose metabolic or perfusion changes. Prospective evaluation of glucose metabolic changes have shown that incident dementia initially may present as a predominant hypometabolic posterior cortical pathology involving the visual association cortex, inferior parietal and temporal regions, the posterior cingulum and precuneus in PD.[52,53] Subsequent progression to dementia is associated with mixed subcortical, including the thalamus and caudate nuclei, and widespread cortical changes that involve the anterior cortices as well.[52,54] Spatial covariance mapping has been particularly useful in providing information regarding the network topography that underlies cognitive dysfunction in PD.[35] Indeed, this approach has revealed a distinct PD cognition-related pattern (PDCP) characterized by metabolic reductions in the medial prefrontal, premotor, and parietal association regions, with relative increases in the cerebellar vermis and dentate nuclei. In cross-sectional analyses, increased PDCP expression is associated with more severe cognitive impairment. PDCP expression is abnormally elevated even in PD patients without evidence of cognitive impairment, and highest in those with dementia.[35] Of note,

PD subjects whose executive function improved with levodopa administration ("responders") also exhibited concurrent reductions in PDCP expression. By contrast, PD subjects showing no cognitive improvement ("non-responders") exhibited no change in PDCP expression with levodopa.[55] Both groups, however, exhibited significant levodopa-mediated reductions in PDRP expression, underscoring the functional distinction between the cognitive and motor networks. These findings together with a recent study on glucose metabolism in groups of patients along the spectrum of parkinsonism to dementia (PD, PDD, DLB, AD) support the notion that glucose metabolism patterns may reflect more the clinical syndrome than the underlying pathology.[56]

An intriguing dual syndrome cognitive hypothesis has been proposed, which posits that the high frequency of fronto-striatal executive dysfunction in PD may relate to common dopaminergic deficits[57,58] and that the development of dementia is associated with more widespread and posterior cortical changes secondary to additional pathologies, including cholinergic deficits.[59,60] Although dopaminergic denervation affects specific cognitive functions in PD,[58] striatal and limbo-frontal dopaminergic changes are present in non-demented PD subjects,[58] but their presence is not sufficient to explain the full development of dementia in PD.[61] In contrast, greater cholinergic denervation is shown consistently in PD dementia compared to PD.[61,62] These observations support a more complex pathophysiological model of interacting dopaminergic (Fig. 1) and cholinergic degenerative (Fig. 3, 4) changes producing cognitive dysfunctions in PD.[63-65] However, imaging studies confirmed as well a significant relationship between *in vivo* measures of elevated cortical and, in particular, striatal β- amyloid deposits (measured with [$^{11}$C]PIB) and greater cognitive impairment in PD.[66,67] However, the risk of having an abnormal [$^{11}$C] PIB PET study in PD with dementia substantially underestimated the risk of abnormal β-deposition in the brain at autopsy in people with PD and dementia.[68,69] Interestingly, β-amyloid deposition measured either in vivo with [$^{11}$C] PIB or at autopsy with immunohistochemistry did not necessarily reflect co-existing AD, since those with dementia due to AD would present marked pathologic deposition of both β-amyloid and tau. Furthermore, the distribution of β-amyloid measured with [$^{11}$C] PIB in PDD has a significantly different pattern in the brain demonstrated by principal components analysis compared to those with AD.[70] Together these data indicate the relevance of β-amyloid brain deposition in PD but suggests that this does not merely reflect the full spectrum of AD pathology.

All of these data demonstrate that neurotransmitter and proteinopathy changes have independent and incremental contributions to the cognitive syndrome in PD.[65] However, other factors (i.e. neuroinflammation) may also play a role. In fact, it has been shown that there may exist a direct relationship between β-amyloid load and levels of microglial activation in PD dementia subjects,[71] suggesting that neuroinflammation may be an early phenomenon, before the dementia onset, and that amyloid along with microglial activation could together contribute not only to the local neuronal dysfunction, but also to the more remote neuronal disconnection.[71] Thus for future studies, there is a need for new ligands for not only neurotransmission, in particular norepinephrine, but also new neuroinflammatory (besides TSPO binding tracers) and proteinopathy targets, especially tracers to visualize neurofibrillary tau and α-synuclein protein aggregates for more comprehensive understanding of the cognitive impairment syndrome in PD.

## Behavioral and Affective Complications in Parkinson's disease

***What molecular imaging has taught us about behavioral spectrum disorders***

There is evidence of a behavioral spectrum disorder ranging from hypo-dopaminergic levels responsible for apathy, anxiety, and depression as described in the withdrawal dopaminergic syndrome to hyper-dopaminergic syndrome including impulse control disorders (ICDs), hallucinations and psychosis.[72,73] Often depression may manifest before the diagnosis of PD, however there is considerable evidence suggesting that this complication can be associated with a more widespread neurodegenerative process. Imaging reports seem to suggest involvement of both dopaminergic and serotonergic systems. While studies using the dopamine transporter radioligand (i.e. TRODAT-1) found significantly higher DAT density in the striatum of depressed PD patients,[74] other investigations with serotonin transporter (i.e. [$^{11}$C]DASB) showed abnormal serotonergic neurotransmission in the raphe nuclei and limbic structures which correlated with depression measures.[75] The serotonergic alteration in depression was confirmed by another PET study using $^{18}$F-MPPF, a selective serotonin 1A receptor antagonist.[76] Apathy may also occur in up to 40% of PD patients and, although clinically distinct from depression, the two are often comorbid.[72] Anatomical and imaging reports have provided evidence that network abnormalities within the prefrontal–striatal circuit can lead to an apathetic behavior. Classically,

apathy is the result of a disruption of 'emotional-affective' mechanisms linked to the ventral striatum, ventromedial prefrontal cortex (PFC) and amygdala.[77] PET studies with D2/D3 receptor antagonist, [11C]-raclopride, have shown several differences in dopaminergic binding and transmission in the mesocorticolimbic system between apathetic and non-apathetic PD patients.[78] [11C]-raclopride binding potential was increased in apathetic PD patients in the orbitofrontal cortex (OFC), cingulate cortex, dorsolateral prefrontal cortex (DLPFC), amygdala as well as in the striatum, implying either (reactive) increase in D2/D3 receptor expression and/or reduction in endogenous synaptic dopamine. Other PET studies with [$^{11}$C]RTI-32, a ligand with affinity to both dopamine and noradrenaline transporters, confirmed that the degree of apathy severity was inversely correlated with [$^{11}$C]RTI-32 binding in the ventral striatum.[79] Taken together, these observations seem to suggest that apathy in PD may result from severe dopamine abnormalities in the mesocorticolimbic system, leading to an impaired emotion reactivity and poor decision-making processes,[77] as also demonstrated in nonhuman primate studies of apathetic behaviors after MPTP.[80,81] However, from more recent evidence, the mechanism of apathy, depression and anxiety in PD may be more complex and may differ according to the stage of the disease. In fact, in de novo PD, serotonergic rather than dopaminergic degeneration appears to play a significant role in this non-motor triad.[82]

While certain behavioral complications are often inherent to the disease process, others are mainly associated with symptomatic treatments. Dopamine agonists for example have been implicated in the development of impulse control disorders (ICDs).[83] Susceptibility to these behavioral addictions is associated with increased striatal dopamine release[84-86] and reduced DAT binding in the ventral striatum.[87] While abnormalities in dopaminergic processing in the ventral striatum are critical for the development of ICDs, prefrontal mechanisms may also play an important inhibitory role in these behaviors. Activation PET studies with $H_2[^{15}O]$ before and after administration of a dopamine agonist in PD with and without gambling behavior found changes in brain areas implicated in impulse control and response inhibition (lateral orbitofrontal cortex, rostral cingulate zone, amygdala).[88] Although the agonist significantly increased regional cerebral blood flow (rCBF) in these areas in healthy subjects, gamblers showed, in contrast, a significant reduction of activity. A subsequent PET study using the extrastriatal dopamine receptor ligand [$^{11}$C]FLB-457[89] found significant abnormalities in D2 receptor binding in the

OFC and anterior cingulate cortex (ACC) in PD patients with PG, thus confirming the role of prefrontal control in the development of ICDs. Similarly, [$^{18}$F]fluorodopa PET has shown abnormalities in the OFC of PD patients with ICDs.[90] In PD patients, deep brain stimulation of the subthalamic nucleus (STN-DBS) may also contribute to certain impulsive behavior associated with high-conflict decisions.[91,92] A regional cerebral blood flow study with $H_2$[$^{15}$O] PET during a Go/NoGo task showed a relationship between motor improvement and response inhibition. In particular, STN-DBS affected response inhibition, as revealed by an increase in commission errors in NoGo trials[91] and stop signal task.[92] These behavioral changes were accompanied by changes in synaptic activity characterized by a reduced activation in the cortical networks associated with proactive and reactive response inhibition. These observations suggest that modulation of STN with DBS, although it improves motor functions, may tend in parallel to favor the appearance of certain impulsive behaviors by acting on mechanisms involved in response initiation and/or selection.[91] However, to date, the impact of DBS (and its interaction with dopamine agonist reduction) on the development of ICD is unclear and still quite controversial, as studies using different approaches have shown conflicting results.[93-95]

Fatigue is a common non-motor symptom in PD. Recent studies have reported that PD patients with higher level of fatigue may show anti-correlated metabolic changes in cortical regions associated with the salience (i.e., right insular region) and default (i.e., bilateral posterior cingulate cortex) networks.[96] Other studies of dopaminergic and serotoninergic function in PD patients with and without fatigue demonstrated a serotoninergic denervation in the basal ganglia and related limbic circuits.[97] PD patients with fatigue had significantly lower SERT binding than patients without fatigue in the basal ganglia structure.[97] Additionally, voxel-based analysis identified reduced dopaminergic activity in caudate and insula, and further SERT reductions in cingulate and amygdala in the fatigue group. All together these findings provide the rationale for treatment strategies aiming to increase brain level of serotonin and serotoninergic transmission as potential treatment of this common complication in PD patients.

## Differentiating atypical Parkinsonisms from PD

***Is molecular imaging helping us?***

To date most molecular imaging studies in parkinsonism have focused on investigating either dopaminergic changes or cerebral blood flow and metabolism. Progressive Supranuclear Palsy (PSP) and Corticobasal Syndrome (CBS) are common forms of atypical parkinsonism (APS), and in early stages, can be sometimes quite difficult to diagnose as they can overlap clinically with PD, and other parkinsonian syndromes including multiple system atrophy (MSA). Previous imaging studies have reported in CBS either asymmetric hypoperfusion or reduced metabolism mainly in the striatum as well as parietal and frontal cortex contralateral to the affected limb.[98] CBS may also be variably associated with asymmetric striatal dopamine denervation (e.g. [99]). Similarly, PSP patients may present with a variable pattern of hypometabolism in the fronto-striatal-thalamic regions depending on the clinical presentation and progression.[98,100] Families with mutations in the progranulin gene presented profound imaging changes of asymmetric hypoperfusion on SPECT and parietal atrophy.[101,102] Subsequent work in CBS identified that hypoperfusion within the left inferior parietal lobule, including the left angular gyrus was associated with more severe ideomotor apraxia. Voxel-based spatial covariance mapping has also been used to identify disease-specific networks for MSA, PSP, and CBD.[103,104] Importantly, an automated logistic regression algorithm based on pattern expression values has been developed to aid in discriminating individuals with idiopathic PD from those with atypical parkinsonian syndromes, and in differentiating among the various forms of APS. This approach had excellent diagnostic specificity in an original data set[105] and in a subsequent validation sample.[106] Other methods such as relevance vector machine analysis have also been used for single case classification with promising results.[107,108] Prospective validation studies are needed before the relative utility of these methods can be determined. Neuroinflammation may play an important role in various atypical parkinsonisms. To date, only a few studies have investigated neuroinflammation in these disorders using mainly first generation of radiotracers, i.e. [$^{11}$C]*(R)*PK11195.[109] While the findings are highly suggestive, they require further confirmation.

Other studies assessed the diagnostic value of dopaminergic tracers using ($^{18}$F)-FP-CIT PET in differentiating PSP and MSA from PD.[110] Compared to PD, PSP and MSA have more prominent dopamine transporter loss in the anterior caudate and ventral putamen, respectively. However, it should be emphasized that the pattern of presynaptic dopaminergic impairment is not felt to

reliably differentiate among various neurodegenerative forms of parkinsonism. Studies in which presynaptic dopamine markers are combined with measures of postsynaptic dopamine receptors may help differentiate PD from atypical parkinsonian syndromes, but not between these various syndromes. Except in a few highly specialized centers, neuroimaging has had a very limited diagnostic value in the differential diagnosis of PSP, CBS and other tauopathies. For this reason, the development of PET radiotracers specific for tau represents one of the most active and challenging areas in molecular imaging.

Several groups have recently reported encouraging results toward the development of selective tau imaging agents.[111,112] [$^{18}$F]T807 (also known as [$^{18}$F]-AV-1451) has been reported having excellent selectivity for paired helical filaments of tau.[111,112] This tracer demonstrated high-affinity and selectivity as well as favorable in-vivo properties, making this a potentially promising candidate as an imaging agent for tau.[111] Another tracer, [$^{11}$C]PBB3, has also been recently applied to human studies, providing PET demonstration of spreading tau pathologies in transition from normal aging to advanced Alzheimer's disease.[113] PET imaging of sporadic 4-repeat tau pathologies in PSP and CBD is currently being conducted, and preliminary data have indicated an increased retention of [$^{11}$C]PBB3 in multiple brain areas, including white matter, in patients with these disorders relative to age-matched controls. Studies of in-vitro binding assays using brain homogenates found that the two tau probes, PBB3 and T807, do not compete with each other for binding sites in PSP tau aggregates. Other investigations with [$^{18}$F]-AV-1451 (i.e. [$^{18}$F]T807) in PSP and CBS have shown preliminary findings with either increase[114] or no retention.[115]

## Future directions of molecular imaging: need for harmonization and multi-center collaboration

Exciting new tracer developments hold the potential for *in vivo* markers of underlying pathology, which is of particular interest for interventions directly targeting protein aggregation. However, with increasingly diverse and sophisticated imaging approaches, it is now becoming more problematic than ever for non-experts to assess the validity and significance of new studies. The scientific community should therefore move toward common standards and harmonization in molecular imaging. Good scientific practice criteria including sample size, correction for

multiple comparisons, correction for the effects of age, motion, and partial volume effects are among the most important issues to address. Anticipating excessive use of the term "biomarker" in imaging studies, we propose to use standardized terminology, which may help in the design future experiments, especially when looking for surrogate markers in interventional studies. In line with the FDA/NIH BEST Resource (2016) propositions, a biomarker is a defined as a characteristic that is measured as an indicator of a biological (pathogenic) process. A diagnostic biomarker should increase diagnostic accuracy for pathological or clinical entities in comparison to clinical judgment alone. In agreement with this definition, certain imaging characteristics may also serve to enrich specific features in a trial population (for example as a target verification tool in case of tau PET). Monitoring biomarkers are measured serially and used to detect a change in the degree or extent of disease. This kind of imaging characteristic may serve as a biomarker that may predict clinical efficacy but not likely to act as a surrogate endpoint since such an imaging biomarker would not reflect unintended side effects. For PD, most valuable biomarkers would certainly be prodromal diagnostic biomarkers and monitoring biomarkers for disease progression at early/prodromal stages. For atypical parkinsonism, there often is a mismatch between clinical and pathological entities (e.g. CBS with AD pathology, PSP pathology with different clinical phenotypes).[116,117] Therefore, diagnostic biomarkers in atypical parkinsonism should not be regarded as diagnostic for a clinical entity, unless the biomarker is pathologically validated. While certainly fruitful, this kind of endeavor only seems feasible in a large multi-center studies strategically focusing on the integration of postmortem information.

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## Authors' Roles

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## Figure Legends

**Figure 1** depicts the ascending nigrostriatal (red), mesolimbic (green), and mesocortical (blue) dopaminergic pathways.

**Figure 2** shows dopaminergic nerve terminal and various PET radiotracers for the assessment of its integrity.

**Figure 3** (top) depicts the distribution of the serotonergic pathways (Yellow), (bottom) depicts the distribution of the cholinergic pathway (Red).

**Figure 4** shows cholinergic (left) and serotonergic (right) nerve terminal and various PET radiotracers for the assessment of their integrity.