

Review

Imaging Biomarkers in Prodromal and Earliest Phases of Parkinson's Disease

Hendrik Theis^{a,b}, Nicola Pavese^{c,d}, Irena Rektorová^{e,f,g} and Thilo van Eimeren^{a,b,*}

^aUniversity of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Nuclear Medicine, Multimodal Neuroimaging Group, Cologne, Germany

^bUniversity of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Neurology, Cologne, Germany

^cAarhus University, Institute of Clinical Medicine, Department of Nuclear Medicine & PET, Aarhus N, Denmark

^dNewcastle University, Translational and Clinical Research Institute, Newcastle upon Tyne, United Kingdom

^eMasaryk University, Faculty of Medicine and St. Anne's University Hospital, International Clinical Research Center, ICRC, Brno, Czech Republic

^fMasaryk University, Faculty of Medicine and St. Anne's University Hospital, First Department of Neurology, Brno, Czech Republic

^gMasaryk University, Applied Neuroscience Research Group, Central European Institute of Technology – CEITEC, Brno, Czech Republic

Accepted 7 January 2024

Abstract. Assessing imaging biomarker in the prodromal and early phases of Parkinson's disease (PD) is of great importance to ensure an early and safe diagnosis. In the last decades, imaging modalities advanced and are now able to assess many different aspects of neurodegeneration in PD. MRI sequences can measure iron content or neuromelanin. Apart from SPECT imaging with Ioflupane, more specific PET tracers to assess degeneration of the dopaminergic system are available. Furthermore, metabolic PET patterns can be used to anticipate a phenoconversion from prodromal PD to manifest PD. In this regard, it is worth mentioning that PET imaging of inflammation will gain significance. Molecular imaging of neurotransmitters like serotonin, noradrenaline and acetylcholine shed more light on non-motor symptoms. Outside of the brain, molecular imaging of the heart and gut is used to measure PD-related degeneration of the autonomous nervous system. Moreover, optical coherence tomography can noninvasively detect degeneration of retinal fibers as a potential biomarker in PD. In this review, we describe these state-of-the-art imaging modalities in early and prodromal PD and point out in how far these techniques can and will be used in the future to pave the way towards a biomarker-based staging of PD.

Keywords: MRI, PET, neuroimaging, Parkinson's disease, progression, diagnosis, prodromal, biomarker

INTRODUCTION

In Parkinson's disease (PD), a biomarker-based diagnosis like in Alzheimer's disease is currently not

established. However, an early and specific diagnosis predicated on a biomarker-based staging in prodromal and early stages of the disease would be of great importance. This would offer the possibility to include patients early in clinical trials and objectively monitor disease progression [1]. Proposals for biomarker-based staging of PD have been previously introduced and follow a similar concept, but differ in terms of consideration of genetic PD and neurode-

*Correspondence to: Thilo van Eimeren, MD, FEAN, University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Nuclear Medicine, Multimodal Neuroimaging Group, Kerpenerstr. 62, 50937 Cologne, Germany. E-mail: thilo.van-eimeren@uk-koeln.de.

generation apart from the nigrostriatal dopaminergic system [2, 3]. In the following, we review the state of the art regarding imaging biomarkers in the prodromal and early stages of PD. Table 1 summarizes these imaging modalities with a special focus on their use in clinical practice. Furthermore, we discuss future perspectives of these biomarkers and the extent to which they can facilitate the way toward a biomarker-based diagnosis and disease staging.

STATE OF THE ART

Magnetic resonance imaging (MRI)

Gray matter (GM) atrophy is a cardinal sign of neurodegeneration which may be depicted by structural MRI using T1-weighted sequences. A typical GM atrophy diagnostic pattern has not yet been conclusively established in PD [4, 5]. More pronounced changes can be observed in PD with manifested cognitive decline [6], with the most consistent early PD-specific pattern of brain atrophy has been identified using deformation based morphometry and partial least squares [7]. The authors described a “PD-specific atrophy network” involving particularly subcortical, but also cortical regions. Individual variation in this atrophy pattern was not only correlated with PD symptoms, but also with CSF biomarkers and predicted scores in the motor and nonmotor domains. Moreover, this network predicted disease trajectories according to another longitudinal study [8]. Notably, using the same methods, a distinct atrophy pattern has been reported in isolated REM sleep behavioral disorder (iRBD) [9]. The same group also showed that cortical regions with greater structural and functional connectivity to the “PD-related atrophy network” demonstrated greater cortical atrophy over the 1-year period [10] hinting at protein spread along the brain’s neuronal connectome.

Changes in white matter fiber integrity can be assessed using single-tensor diffusion imaging (DTI). The two most common DTI parameters used are mean diffusivity (MD) and fractional anisotropy (FA). While decreased FA and increased MD are usually found in PD and interpreted as decreased fiber integrity [11], other possible underlying mechanisms include e.g. axonal degeneration, demyelination, and larger axonal diameter. DTI changes in substantia nigra (SN) have been rather controversial [12]. The assumption of the Gaussian distribution of water diffusion is the greatest limitation of DTI. Diffusion kurtosis imaging (DKI) as an extension of

DTI can overcome this limitation. In a TNWT-61 genetic mouse model of PD, increased kurtosis in the thalamus was positively correlated with the total alpha-synuclein signal in the region [13]. Using this method in humans, the same group demonstrated increased diffusion kurtosis in the SN and the motor and premotor cortices in early PD [14]. Conversely, kurtosis decreases in cortical GM, decreased fiber integrity as assessed by FA and MD, and gross atrophy as assessed by DBM were dominant features of cognitively impaired PD patients as compared to early cognitively normal PD. Taken together, early increases of kurtosis in early PD likely reflect increased hindrance to water diffusion potentially caused by α -synuclein deposition. In more advanced PD with cognitive impairment the changes are in the opposite direction likely reflecting neurodegeneration.

A two-compartment diffusion model, free-water imaging, was implemented in iRBD [15] and in early PD [16], finding that the free water component may be increased in the posterior SN as compared to HC. This indicator of early neurodegeneration has been interpreted as likely reflecting neuro-inflammation-related oedema, blood-brain barrier disruption or atrophy (Fig. 1, FW).

Another MRI technique depicts iron in the brain and particularly in the substantia nigra pars compacta (SNc). It uses iron sensitive sequences such as T2* relaxometry or susceptibility weighted imaging (SWI) detecting an oval hyperintensity within the dorsolateral SN which constitutes the nigrosome-1. This dorsal nigral hyperintensity (DNH), also called the ‘swallow tail sign’, is detectable in individual healthy controls (HC), but is reduced or lost in PD due to increased iron deposition in this region [17] (see Fig. 1, DNH). Absence of DNH has been shown already in about two thirds of iRBD patients [18]. Unfortunately, the method currently only provides a qualitative (‘yes’ or ‘no’) answer and not a quantification of pathology, as with presynaptic dopaminergic imaging (see below).

Quantitative susceptibility mapping (QSM) enables quantification of iron in the brain, congruent with histologic validation of iron content [19]. It is a more sensitive measure than R2* relaxometry in terms of detecting nigral iron increase due to PD, as QSM accounts better for local tissue susceptibility than R2* [20]. Changes in SN have already been described in iRBD and may be present years before the onset of PD [21]. QSM may also be assessed in the parcellated striatum—one study reports that

Table 1
Imaging Biomarkers assessing neurodegeneration in early PD and their use in clinical routine

Modality	Assessment of PD-related neurodegeneration	Availability and clinical use
Structural MRI	<ul style="list-style-type: none"> • Excluding secondary parkinsonism. • Detection of cerebral atrophy patterns. 	<ul style="list-style-type: none"> • Broadly available and established in clinical routine. • Included in MDS PD criteria. • Broadly available and established in clinical routine.
Iron-sensitive MRI T2*, SWI, QSM	<ul style="list-style-type: none"> • Quantifiable iron depositions in PD. • Swallow Tail Sign (dichotomous). 	<ul style="list-style-type: none"> • T2* and SWI are broadly available, QSM not. • Not established in clinical routine.
Neuromelanin-sensitive MRI	<ul style="list-style-type: none"> • Quantifiable degeneration of substantia nigra and locus coeruleus. 	<ul style="list-style-type: none"> • Not broadly available, not established in clinical routine.
Diffusion MRI	<ul style="list-style-type: none"> • Changes in WM integrity/ GM microstructure 	<ul style="list-style-type: none"> • Not broadly available, not established in clinical routine for diagnostic purposes.
Functional MRI	<ul style="list-style-type: none"> • Task-based activations, functional connectivity 	
DaT-SPECT	<ul style="list-style-type: none"> • Quantifiable degeneration of dopaminergic terminals. 	<ul style="list-style-type: none"> • Established in clinical routine.
DOPA-PET PE2I-PET		<ul style="list-style-type: none"> • Included in MDS PD criteria. ◊ DaT-SPECT: Broadly available. ◊ DOPA: Available. ◊ PE2I: Limited availability.
FDG-PET	<ul style="list-style-type: none"> • PD-related metabolic patterns. 	<ul style="list-style-type: none"> • Broadly available, but not established in clinical routine.
TSPO-PET	<ul style="list-style-type: none"> • Imaging of microglia activation (inflammation). 	<ul style="list-style-type: none"> • Not broadly available, not established in clinical routine.
Acetylcholine-, Serotonin-, Noradrenalin-PET	<ul style="list-style-type: none"> • Quantifiable degeneration of the cholinergic, serotonergic and noradrenergic system. 	<ul style="list-style-type: none"> • Not broadly available, not established in clinical routine.
MIBG-Scintigraphy/ SPECT	<ul style="list-style-type: none"> • Quantifiable sympathetic denervation of the heart. 	<ul style="list-style-type: none"> • Available and established in clinical routine. • Used mostly for differential diagnosis (PD vs. MSA). • Supportive criterium in MDS PD criteria.
OCT	<ul style="list-style-type: none"> • Quantifiable degeneration of retinal fibers. 	<ul style="list-style-type: none"> • Available. Not established in clinical routine.

changes in the caudal motor striatum can be identified at the single-subject level with good accuracy [22] (see Fig. 1, QSM).

Another promising MRI method is neuromelanin sensitive (NMS) MRI. It is well known that dopaminergic neurons in SNc and noradrenergic neurons in locus coeruleus (LC) contain the pigment neuromelanin. The source of NM contrast is the NM binding to iron, which is paramagnetic. Therefore, reduced NMS signal can be observed in the posterolateral motor areas of SN in early PD and iRBD compared to HC with good classification accuracy [23], and even 5–6 years before the onset of symptoms of PD (see Fig. 1, NMS) [21]. LC-MRI signal loss could also be found at early PD and in iRBD [24].

We find it important to at least briefly mention functional MRI. A recent study [25] showed global (whole-brain) network changes in a large sample of early mild drug naïve PD patients as compared to HC. At the connectome level, the authors described impaired basal ganglia connections particularly to

the sensorimotor, default mode, and visual networks in PD, which fits well with the connectome spreading model of brain pathology. Based on more recent literature, it seems that the default mode network ‘enhancement or inhibition’ in PD is driven by specific phenotypes such as hallucinations and cognitive impairment, respectively [26, 27].

Imaging of the presynaptic dopaminergic system

It is assumed that the motor symptoms of PD occur when about 50% of nigrostriatal dopaminergic neurons are degenerated. However, newer results indicate that the loss of dopaminergic terminals seems to be more around 30% after adjusting for age [28]. Imaging of the dopaminergic system therefore offers the possibility to assess neurodegeneration even in pre-clinical and prodromal phases of PD and represents a powerful biomarker in these earliest disease stages.

Several different tracers for positron emission tomography (PET) and single photon emission

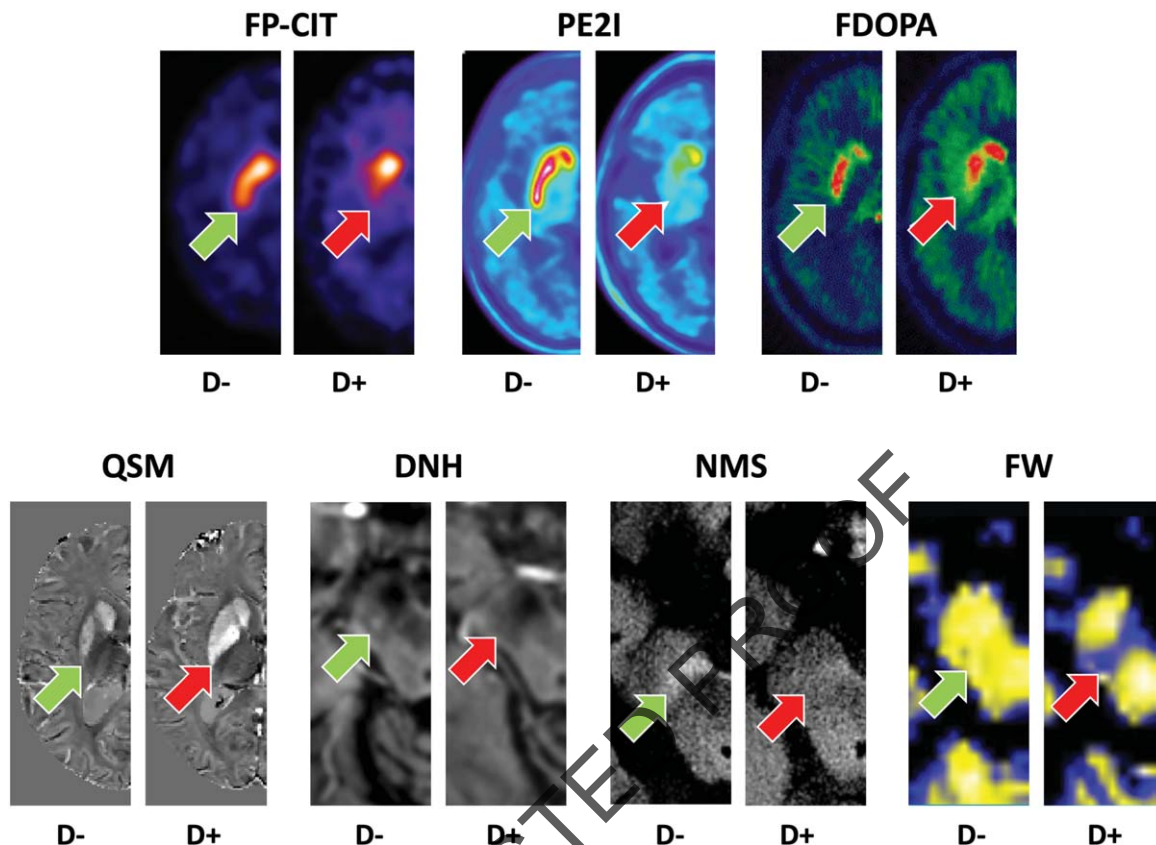


Fig. 1. Imaging Biomarkers of Dopaminergic Degeneration in PD. Various PET or MRI modalities can capture dopaminergic degeneration (see Table 1 for availability and use in clinical routine). Examples of normal (D-) and pathological (D+) findings. Red arrows indicate abnormality. Normal findings pointed out with green arrow. All images are in axial orientation. FP-CIT: dopamine transporter SPECT using $[^{123}\text{I}]\text{FP-CIT}$; PE2I: dopamine transporter PET using $[^{18}\text{F}]\text{FE-PE2I}$; FDOPA: presynaptic dopamine turnover PET using $[^{18}\text{F}]\text{DOPA}$; QSM: Quantitative susceptibility mapping MRI of iron load the striatum. DNH: Dorsal nigral hyperintensity in SWI or T2* MRI, a.k.a. 'swallow tail sign'. NMS: Neuromelanin-sensitive MRI of the substantia nigra; Courtesy Stephane Lehericy, Rahul Gaurav (Paris Brain Institute, France). FW: Free water diffusion MRI of the posterior substantia nigra; Courtesy David Vaillancourt (University of Florida, USA).

computed tomography (SPECT) of the presynaptic dopaminergic system are available. The most commonly used in clinical routine is SPECT imaging of the dopamine transporter (DaT) with $[^{123}\text{I}]\text{FP-CIT}$ (see Fig. 1, FP-CIT). However, the novel highly selective DaT PET-tracers $[^{18}\text{F}]\text{FE-PE2I}$ represents a promising alternative (see Fig. 1, PE2I). Apart from DaT imaging, quantification of presynaptic dopamine turnover with $[^{18}\text{F}]\text{DOPA}$ offers another possibility to measure nigrostriatal dopaminergic degeneration [29] (see Fig. 1, FDOPA).

DaT imaging can be visually interpreted and additionally confirmed by a semiquantitative analysis. A z-score below -2 for striatal binding ratios has been defined as an abnormal threshold during semiquantitative analysis [30, 31]. However, especially in prodromal PD, less conservative thresholds might be necessary to detect the earliest phases of nigros-

triatal degeneration. A recent study could show that for differentiating PD a z-score cut-off below -1.27 of the posterior putamen of the more affected hemisphere shows the highest accuracy [32]. However, the type of tracer and imaging modality also seem to play an important role in early and prodromal PD. With the higher resolution of PET and the higher selectivity of $[^{18}\text{F}]\text{FE-PE2I}$ for DaT a higher diagnostic accuracy can be achieved as compared to $[^{123}\text{I}]\text{FP-CIT}$ [33]. It could be shown with $[^{18}\text{F}]\text{DOPA}$ -PET and functional MRI that dopaminergic degeneration in early PD follows a somatotopic pattern, that is, an upper limb-related degeneration in the putamen and a progression over 2 years to the less affected hemisphere [34]. In this regard it is worth mentioning the interesting link between DaT and DOPA imaging. Asymptomatic patients with familial PD showed a downregulation of DaT while $[^{18}\text{F}]\text{DOPA}$ -

PET revealed an upregulated dopamine turnover [35, 36].

A very important topic is the association of DaT imaging in prodromal PD with phenoconversion to clinical PD. A longitudinal study showed that patients with iRBD with a DaT-specific putamen binding ratio below 48% had significantly higher short-term risk of developing PD [37]. Another study could demonstrate that a putaminal uptake decrease of at least 25% predicted Parkinsonism after 3 years [38]. Interestingly, it could be shown that those iRBD patients with DaT reduction did not differ in demographic or clinical features at baseline [39]. However, DaT SPECT signal was correlated with REM sleep without atonia measures [40]. To conclude, iRBD patients with reduced DaT binding have a high risk for (earlier) phenoconversion to clinical PD independent of other demographic or clinical features. It could be shown that PD patients with iRBD show, compared to PD without iRBD, a more symmetric reduction of dopaminergic terminals [41]. However, this finding was not supported in data of the PPMI cohort [5]. Interestingly, another study indicated that right-handed patients with iRBD have a left-hemispheric predominance of subclinical nigrostriatal dysfunction, which was also shown in clinically established PD [42]. Due to these inconsistent findings, the pattern of DaT reduction in iRBD, i.e., more symmetric or lateral reduction, warrants further investigations. In addition to iRBD, idiopathic hyposmia (iH) is also a prodromal marker of PD. Patients with iH who had abnormal DaT-SPECT at baseline and those who developed abnormal DaT-SPECT at follow-up had the highest rate of phenoconversion to PD [43, 44]. Olfactory dysfunction itself showed a moderate correlation with dopamine turnover in the putamen [45].

It should be emphasized that the negative predictive value of DaT imaging is greater than the positive predictive value, which means that a normal DaT-SPECT practically excludes conversion to clinical PD in the near future [46, 47]. Hence, DaT-SPECT might gain great importance as a surrogate endpoint in clinical trials in prodromal PD [1]. In this regard, it is worth mentioning that imaging of vesicular monoamine transporter 2 with [18F]AV-133 might be an interesting biomarker to monitor the disease progression during therapeutic trials [48]. We want to point out that dopaminergic imaging can be influenced by some drugs, which includes the risk of false-positive results [49, 50]. Importantly, dopamine replacement therapy has no impact on DaT binding [50].

Imaging of inflammation–translocator protein

There is robust evidence that neuroinflammation plays a significant role in the development and progression of PD and other neurodegenerative disorders. The inflammatory response of the brain is crucially driven by activation of microglia [51]. Neuroinflammation can be demonstrated *in vivo* with PET ligands targeting the 18 kDa translocator protein (TSPO). TSPO is mainly expressed in the mitochondrial outer membrane of microglia and its expression is greatly increased when microglia are activated. Several teams have reported widespread increases in microglial activation in PD [52]. Even the earliest patients have shown widespread microglia activation targeting the brainstem, basal ganglia, and frontal cortex [53]. The presence of microglial activation in brain regions susceptible to Lewy body pathology has also been detected in patients with iRBD [54, 55], and in asymptomatic carriers of genetic mutations linked to PD (*GBA1* and *LRRK2* mutations) [56, 57]. In patients with iRBD, increased load of microglial activation in the substantia innominata, the major source of cholinergic input to the cortex, correlated with cortical cholinergic dysfunction measured with [11C]Donepezil [58]. In *GBA1* mutations carriers, interestingly, microglial activation in the SN correlated with hyposmia and seemed to precede the loss of striatal dopaminergic terminals measured with [18F]DOPA-PET.

Metabolic imaging

Metabolic imaging in preclinical and prodromal PD is commonly done with [18F]FDG-PET and can be used to evaluate disease related pathological patterns.

A recent study compared the iRBD-related pattern with the established PD-related pattern. The two patterns overlapped and the iRBD-related pattern was significantly expressed in PD patients compared to controls [59]. In a further step, the Scaled Subprofile Model and Principal Component Analysis (SSM-PCA) was used to predict phenoconversion of iRBD to PD by deriving a conversion pattern to PD. This pattern showed a sensitivity of 87% and a specificity of 72% discriminating converters from nonconverters and this conversion pattern was validated in an independent cohort [60, 61]. A multimodal study that combined PD-related pattern expression, DaT imaging, and olfactory tests could show that pattern expression was higher in iRBD with iH and in

patients with reduced DaT binding [59]. The expression of the suprathreshold PD-related FDG pattern and an increase in this pattern over time may indicate a greater short-term risk of phenoconversion to PD [62]. Interestingly, short-term conversion was explicitly driven by occipital hypometabolism at baseline in iRBD [63]. Additionally, hypometabolism in occipital and parietal areas was associated with mild cognitive impairment (MCI) in iRBD, which is also a predictor for phenoconversion to PD [64]. Additionally, the perfusion phase of other PET or SPECT tracers can be used to acquire equivalent information to FDG-PET [29, 65].

Imaging of acetylcholine

Posterior cortical cholinergic degeneration is prominent already in non-demented, de-novo Parkinson's disease [66, 67], with evidence of a posterior to anterior progression over time [68]. GBA-mutation carriers with PD, who are at greater risk for early cognitive decline, express a more widespread reduction of cortical cholinergic denervation despite normal cognition [69]. Increasing cortical cholinergic dysfunction specifically characterised a group of iRBD patients with cognitive decline in another study with a small sample [70]. Grey matter density of the basal nucleus of Meynert, the main origin of cortical cholinergic innervation, was significantly reduced in 35 iRBD patients and reduction was associated with poorer performance in a working memory task [71].

Imaging of serotonin

The degeneration of serotonergic innervation in striatal and extrastriatal brain regions is considered an early feature of PD [72]. However, people living with PD but without depression, apathy, or anxiety may have a relatively preserved serotonergic system [73]. Loss of serotonergic innervation of the limbic cortico-striatal system in PD has been linked to the presence and severity of apathy and anxiety associated with lower [74]. Apathy in PD was associated with lower serotonin transporter binding in the basal ganglia [75]. Serotonin transporter signal integrity in the dorsal raphe nucleus was shown to correlate with apathy severity in prodromal PD [76]. Overall, it seems that serotonergic deficits, which can be monitored with brain imaging, may precede motor deficits and identify those patients with a risk of apathy, anxiety, and depression.

Imaging of noradrenaline NA

Currently, the integrity of the noradrenergic system is studied either analyzing neuromelanin-dependent MRI signal of the small locus coeruleus— the principal origin of noradrenergic neurons of the brain, or using PET radioligands targeting receptors or transporters of noradrenalin. Interestingly, studies have rather consistently shown a dissociation of locus coeruleus integrity as measured with MRI and integrity of noradrenergic cortical terminals using PET [77, 78]. A widespread cortical (motor cortex and insula) and subcortical (thalamus and putamen) reduction of NA receptors has very recently been described [78]. NA transporters do not appear to be dramatically reduced in prodromal PD, with some evidence for moderately reduced availability of NA transporters in the sensorimotor cortex and the thalamus [79]. In general, there is not much evidence for a distinctive role for NA imaging biomarkers in prodromal or early PD.

Imaging of heart and gut

It is now well accepted that there is severe neuronal pathology in PD also outside the brain, mostly affecting the autonomous nervous system of the heart (noradrenergic) and gut (cholinergic) [80]. It has been speculated that pathology may in fact enter the brain via the vagus and the olfactory nerve [81]. (Dys-)function of the enteric nervous system can be objectified by imaging either directly with transmitter imaging (e.g., using [11C]-donepezil) or indirectly and less specific using enteric passage times for radiologic markers. Colonic, but not gastric transfer times seem to be a sensitive marker of enteric autonomous dysfunction in the prodromal and early PD phase [82, 83].

To measure the sympathetic denervation of the heart, SPECT or scintigraphy with iodine radio-labelled MIBG are established methods. Despite relevant shortcomings of the method, it has been used to subcategorize PD into two to three subcategories [84, 85]. The dichotomy of a “brain-first” (no sign of sympathetic denervation at diagnosis) and “body-first” (sympathetic denervation at diagnosis) subtype has received a lot of attention, but is not uniformly supported [5, 86]. Although cardiac imaging is not the only factor that differs between these hypothetical subtypes, it is considered the most critical distinction around the time of PD diagnosis [84]. Interestingly, more severe sympathetic dener-

vation may also differentiate GBA-PD from sporadic PD [87]. Using a clustering approach including cardiac MIBG imaging, another group recently defined a cardio-cortical and a dopamine dominant type [84]. The finding that plasma α -synuclein levels correlated with cardiac sympathetic denervation, but not with nigrostriatal degeneration may also be seen as adding to the circumstantial evidence for a relative dissociation between “peripheral” and “central” PD [88]. Critically, when using this imaging method, it should be considered that drug interactions can significantly influence MIBG uptake, see also [89, 90].

Optical coherence tomography (OCT)

Ophthalmological complaints are common in PD and often precede the onset of motor symptoms. Retinal impairment has also been described in PD from the very early stages of the disease [91]. OCT provides accurate measurements of mean macular thickness (MMT) and thinning of the peripapillary retinal nerve fiber layer (RNFL). In general, these studies have shown that patients with PD have a significant reduction in RNFL and MMT compared to HC, although other studies have failed to find any difference [92, 93].

A recent study in iRBD patients has shown that RNFL and most macular retinal layers were significantly thinner compared to HC. Interestingly, the values observed in iRBD were between those observed in PD and HC [94]. Conversely, another study found no OCT abnormalities in PD patients with LRRK2 mutation and LRRK2 non-manifesting carriers, suggesting that LRRK2-PD could be distinguished from idiopathic PD by absent or less retinal nerve involvement even at the prodromal stage [95].

FUTURE PERSPECTIVES

For MRI biomarkers, as for all other modalities, it will be interesting to bring them into the perspective of the suggested biomarker-based definition of PD [2, 3]. While the traditional domain for MRI could be seen as the evaluation of neurodegeneration *per se*, specific evaluation of biomarkers signaling degeneration of the dopaminergic system is now more relevant than ever. Moreover, imaging will be needed to visualize early spatial brain alterations related to neurodegeneration and brain compensation. To achieve this, harmonization of data acquisition and analytical approaches is warranted for

the use of QSM, NMS, fMRI, and various diffusion methods in clinical practice. Moreover, MRI has also potential to study pathophysiological mechanisms of toxic protein spreading [96]. MRI techniques not only have potential as preclinical PD biomarkers, but more work is needed to prove their utility in predicting early disease phenocconversion, monitoring specific PD symptoms with time, and predicting disease clinical subtypes and disease trajectories. Although some works suggest utility of the above-mentioned MRI methods in, e.g., early differential diagnosis of PD versus atypical parkinsonian syndromes [97], machine learning and AI approaches will have to be further corroborated.

Dopaminergic imaging can already be used as an endpoint reflecting neurodegeneration of the nigrostriatal dopaminergic system in the preclinical and prodromal phases of PD. A previous proposal elaborated the role of aggregated α -synuclein in CSF as well as imaging of dopaminergic degeneration as promising biomarkers for a staging system in PD [2]. Along these lines, dopaminergic imaging represents a valuable tool for biomarker-based staging, where patients with aggregated α -synuclein (S) in absence of substantial dopaminergic (D) system degeneration (S + D⁻) can be identified. This group might represent a promising candidate for therapeutic trials against α -synuclein and subsequent neurodegeneration. In addition, in some patients with iRBD, dopamine transporter density stays normal for a longer time than in others. This indicates underlying factors that prevent or promote nigrostriatal degeneration, which would be imperative to investigate as therapeutic targets.

Future imaging studies investigating neuroinflammation should aim to clarify the actual nature of microglia activation in prodromal PD, as there is evidence that there might be two peaks of microglial activation: an early peak with a protective function and a later pro-inflammatory peak [98]. Therefore, it is crucial to understand when and which subtype of microglia is relevant in prodromal stages. Imaging neuroinflammation has provided a rationale for the trial of anti-inflammatory agents to prevent the progression of iRBD to parkinsonian syndromes. In this line, a Phase 2a study in participants with iRBD (ClinicalTrials.gov Identifier: NCT05904717) will start soon to evaluate whether 12-week treatment with PXS-4728, a very potent and highly selective inhibitor of the enzyme Semicarbazide-Sensitive Amine Oxidase (SSAO), can reduce levels of microglia activation, as measured by PET.

FDG-PET currently has already demonstrated in a research context that a concrete metabolic pattern may indicate iRBD patients at immediate risk for phenoconversion. Further investigation is needed for the relevance of occipital hypometabolism in iRBD patients. It is necessary to disentangle whether this is more relevant as a biomarker for phenoconversion to PD or for cognitive decline.

Cortical cholinergic denervation has been implicated with the development of dementia in PD. However, while there is some evidence for it being a necessary precursor to dementia in PD, the evidence is far from conclusive. It seems clear that early-stage PD already comes with significant loss of cholinergic input to the posterior cortex, but we need to have a better idea about when this process starts in the prodromal phase and how strongly cholinergic denervation in certain parts of the brain is associated with consecutive cognitive decline. If this can be firmly established, imaging biomarkers of cholinergic denervation may ultimately serve as a surrogate endpoint for the development of dementia in PD.

It seems clear that serotonergic denervation is involved in non-motor symptoms such as apathy and anxiety. As the disease develops, serotonergic innervation may also significantly contribute to motor phenomena, including dyskinesias. However, it is currently unclear, what role imaging biomarkers of the serotonin system may play in the prodromal or early PD phase.

Cortical and subcortical noradrenergic innervation plays a physiological role in many motor and non-motor functions that are affected in PD. However, it has yet to be established, how NA imaging biomarkers can be used to monitor PD in the prodromal or early stage.

There is evidence for great heterogeneity in when cardiac imaging is pathologic during the course of PD, which certainly questions the utility of this biomarker as a surrogate endpoint in clinical trials. However, when only considering prodromal PD as characterized by iRBD, there is some evidence that a pathological MIBG may almost always precede pathological DaT SPECT and may therefore be considered as a non-clinical milestone in the prodromal disease progression [99].

It is intriguing that OCT could potentially be used as a simple non-invasive biomarker of PD pathology. Future studies will also be needed to investigate the possible relationship between structural changes in the retina and disease severity or time to phenoconversion to motor PD in prodromal cases to understand

whether OCT can also be used to monitor disease progression.

Currently, there is no validated PET tracer for α -synuclein in PD. For a detailed review on imaging of α -synuclein, see [29]. The tracer [18F]ACI-12589 represents a promising candidate for imaging of α -synuclein in MSA, but showed only very limited binding in PD [100]. In the light of current developments around α -synuclein seeding assays in CSF [101] and the potential use of α -synuclein PET tracers [100], a biomarker-based disease staging in terms of α -synuclein and (dopaminergic) neurodegeneration may be possible in the near future [2].

CONCLUSION

Plenty of imaging methods are available to assess and quantify different facets of neurodegeneration in PD. Currently, biomarkers that may serve as an endpoint in clinical trials surrogating for the therapeutic effect on the development of motor symptoms in the prodromal phase are at the center of interest. While there are many interesting candidates, DaT-SPECT currently is the most broadly available, most established in clinical routine and most validated biomarker for this use case. However, in light of a biomarker-based definition of “neural synucleinopathy” as an entity, biomarkers capturing the advent of other disease features (e.g., dementia) remain important to develop and validate.

ACKNOWLEDGMENTS

The authors have no acknowledgments to report.

FUNDING

This study was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID C03 – 431549029 to TvE. HT was supported by the Cologne Clinician Scientist Program (CCSP) / Faculty of Medicine / University of Cologne. Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) (Project No. 413543196). IR has been supported by the European Union-Next Generation EU: project no. LX22NPO5107 (MŠMT) and by the grant AZV no. NU20-04-00294 of the Czech Ministry of Health.

CONFLICT OF INTEREST

TvE received or receives honoraria for consulting or in advisory roles from Lundbeck Foundation, Lundbeck Pharma, Orion Pharma, GT Gain Therapeutics SA.

REFERENCES

- [1] van Eimeren T, Antonini A, Berg D, Bohnen N, Ceravolo R, Drzezga A, Höglinger GU, Higuchi M, Lehericy S, Lewis S, Monchi O, Nestor P, Ondrus M, Pavese N, Peralta MC, Piccini P, Pineda-Pardo JÁ, Rektorová I, Rodríguez-Oroz M, Rominger A, Seppi K, Stoessl AJ, Tessoro A, Thobois S, Kaasinen V, Wenning G, Siebner HR, Strafella AP, Rowe JB (2019) Neuroimaging biomarkers for clinical trials in atypical parkinsonian disorders: Proposal for a Neuroimaging Biomarker Utility System. *Alzheimers Dement (Amst)* **11**, 301-309.
- [2] Chahine LM, Merchant K, Siderowf A, Sherer T, Tanner C, Marek K, Simuni T (2023) Proposal for a biologic staging system of Parkinson's disease. *J Parkinsons Dis* **13**, 297-309.
- [3] Höglinger GU, Adler CH, Berg D, Klein C, Outcero TF, Poewe W, Postuma R, Stoessl J, Lang AE (2023) Towards a biological definition of Parkinson's disease. *Preprints* **2023**, 2023040108. <https://doi.org/10.20944/preprints202304.0108.v1> [Preprint]. Posted April 7, 2023.
- [4] Krajcovicova L, Klobusiakova P, Rektorova I (2019) Gray matter changes in Parkinson's and Alzheimer's disease and relation to cognition. *Curr Neurol Neurosci Rep* **19**, 85.
- [5] Banwinkler M, Dzialas V, Parkinson's Progression Markers Initiative, Hoening MC, van Eimeren T (2022) Gray matter volume loss in proposed brain-first and body-first Parkinson's disease subtypes. *Mov Disord* **37**, 2066-2074.
- [6] Rektorova I, Biundo R, Marecek R, Weis L, Aarsland D, Antonini A (2014) Gray matter changes in cognitively impaired Parkinson's disease patients. *PLoS One* **9**, e85595.
- [7] Zeighami Y, Fereshtehnejad S-M, Dadar M, Collins DL, Postuma RB, Mišić B, Dagher A (2019) A clinical-anatomical signature of Parkinson's disease identified with partial least squares and magnetic resonance imaging. *Neuroimage* **190**, 69-78.
- [8] Fereshtehnejad S-M, Zeighami Y, Dagher A, Postuma RB (2017) Clinical criteria for subtyping Parkinson's disease: Biomarkers and longitudinal progression. *Brain* **140**, 1959-1976.
- [9] Rahayel S, Postuma RB, Montplaisir J, Mišić B, Tremblay C, Vo A, Lewis S, Matar E, Ehgoetz Martens K, Blanc F, Yao C, Carrier J, Monchi O, Gagnon J-F (2021) A prodromal brain-clinical pattern of cognition in synucleinopathies. *Ann Neurol* **89**, 341-357.
- [10] Yau Y, Zeighami Y, Baker TE, Larcher K, Vainik U, Dadar M, Fonov VS, Haggmann P, Griffa A, Mišić B, Collins DL, Dagher A (2018) Network connectivity determines cortical thinning in early Parkinson's disease progression. *Nat Commun* **9**, 12.
- [11] Atkinson-Clement C, Pinto S, Eusebio A, Coulon O (2017) Diffusion tensor imaging in Parkinson's disease: Review and meta-analysis. *Neuroimage Clin* **16**, 98-110.
- [12] Schwarz ST, Abaei M, Gontu V, Morgan PS, Bajaj N, Auer DP (2013) Diffusion tensor imaging of nigral degeneration in Parkinson's disease: A region-of-interest and voxel-based study at 3 T and systematic review with meta-analysis. *Neuroimage Clin* **3**, 481-488.
- [13] Khairnar A, Ruda-Kucerova J, Szabó N, Drazanova E, Arab A, Hutter-Paier B, Neddens J, Latta P, Starcuk Z, Rektorova I (2017) Early and progressive microstructural brain changes in mice overexpressing human α -synuclein detected by diffusion kurtosis imaging. *Brain Behav Immun* **61**, 197-208.
- [14] Sejnoha Minsterova A, Klobusiakova P, Pies A, Galaz Z, Mekyska J, Novakova L, Nemcova Elfmalkova N, Rektorova I (2020) Patterns of diffusion kurtosis changes in Parkinson's disease subtypes. *Parkinsonism Relat Disord* **81**, 96-102.
- [15] Zhou L, Li G, Zhang Y, Zhang M, Chen Z, Zhang L, Wang X, Zhang M, Ye G, Li X, Chen S, Li B, Wei H, Liu J (2021) Increased free water in the substantia nigra in idiopathic REM sleep behaviour disorder. *Brain* **144**, 1488-1497.
- [16] Ofori E, Pasternak O, Planetta PJ, Burciu R, Snyder A, Febo M, Golde TE, Okun MS, Vaillancourt DE (2015) Increased free water in the substantia nigra of Parkinson's disease: A single-site and multi-site study. *Neurobiol Aging* **36**, 1097-1104.
- [17] Barber TR, Klein JC, Mackay CE, Hu MTM (2017) Neuroimaging in pre-motor Parkinson's disease. *Neuroimage Clin* **15**, 215-227.
- [18] De Marzi R, Seppi K, Högl B, Müller C, Scherfler C, Stefani A, Iranzo A, Tolosa E, Santamaria J, Gizewski E, Schocke M, Skalla E, Kremser C, Poewe W (2016) Loss of dorsolateral nigral hyperintensity on 3.0 tesla susceptibility-weighted imaging in idiopathic rapid eye movement sleep behavior disorder. *Ann Neurol* **79**, 1026-1030.
- [19] Sun H, Walsh AJ, Lebel RM, Blevins G, Catz I, Lu J-Q, Johnson ES, Emery DJ, Warren KG, Wilman AH (2015) Validation of quantitative susceptibility mapping with Perls' iron staining for subcortical gray matter. *Neuroimage* **105**, 486-492.
- [20] Prange S, Metereau E, Thobois S (2019) Structural imaging in Parkinson's disease: New developments. *Curr Neurol Neurosci Rep* **19**, 50.
- [21] Biondetti E, Santin MD, Valabrègue R, Mangone G, Gaurav R, Pyatigorskaya N, Hutchison M, Yahia-Cherif L, Villain N, Habert M-O, Arnulf I, Leu-Semenescu S, Dodet P, Vila M, Corvol J-C, Vidailhet M, Lehericy S (2021) The spatiotemporal changes in dopamine, neuromelanin and iron characterizing Parkinson's disease. *Brain* **144**, 3114-3125.
- [22] Alushaj E, Hemachandra D, Kuurstra A, Menon RS, Ganjavi H, Sharma M, Kashgari A, Barr J, Reisman W, Khan AR, MacDonald PA (2023) Subregional analysis of striatum iron in Parkinson's disease and rapid eye movement sleep behaviour disorder. *Neuroimage Clin* **40**, 103519.
- [23] Pavese N, Tai YF (2018) Nigrosome imaging and neuromelanin sensitive MRI in diagnostic evaluation of parkinsonism. *Mov Disord Clin Pract* **5**, 131-140.
- [24] Galgani A, Lombardo F, Della Latta D, Martini N, Bonuccelli U, Fornai F, Giorgi FS (2020) Locus coeruleus magnetic resonance imaging in neurological diseases. *Curr Neurol Neurosci Rep* **21**, 2.
- [25] De Micco R, Agosta F, Basaia S, Siciliano M, Cividini C, Tedeschi G, Filippi M, Tessoro A (2021) Func-

- tional connectomics and disease progression in drug-naïve Parkinson's disease patients. *Mov Disord* **36**, 1603-1616.
- [26] Bejr-Kasem H, Pagonabarraga J, Martínez-Horta S, Sampedro F, Marín-Lahoz J, Horta-Barba A, Aracil-Bolaños I, Pérez-Pérez J, Ángeles Botí M, Campolongo A, Izquierdo C, Pascual-Sedano B, Gómez-Ansón B, Kulisevsky J (2019) Disruption of the default mode network and its intrinsic functional connectivity underlies minor hallucinations in Parkinson's disease. *Mov Disord* **34**, 78-86.
- [27] Wolters AF, van de Weijer SCF, Leentjens AFG, Duits AA, Jacobs HIL, Kuijff ML (2019) Resting-state fMRI in Parkinson's disease patients with cognitive impairment: A meta-analysis. *Parkinsonism Relat Disord* **62**, 16-27.
- [28] Cheng H-C, Ulane CM, Burke RE (2010) Clinical progression in Parkinson disease and the neurobiology of axons. *Ann Neurol* **67**, 715-725.
- [29] Prange S, Theis H, Banwinkler M, van Eimeren T (2022) Molecular imaging in parkinsonian disorders-what's new and hot? *Brain Sci* **12**, 1146.
- [30] Brogley JE (2019) DaTQUANT: The future of diagnosing Parkinson disease. *J Nucl Med Technol* **47**, 21-26.
- [31] Pencharz DR, Hanlon P, Chakravarty R, Navalkisoor S, Quigley A-M, Wagner T, Wagner T (2014) Automated quantification with BRASS reduces equivocal reporting of DaTSCAN (123I-FP-CIT) SPECT studies. *Nucl Med Rev Cent East Eur* **17**, 65-69.
- [32] Lanfranchi F, Arnaldi D, Miceli A, Mattioli P, D'Amico F, Raffa S, Donegani MI, Chiola S, Massa F, Pardini M, Di Raimondo T, Sambuceti G, Bauckneht M, Nobili F, Morbelli S (2023) Different z-score cut-offs for striatal binding ratio (SBR) of DaT SPECT are needed to support the diagnosis of Parkinson's disease (PD) and dementia with Lewy bodies (DLB). *Eur J Nucl Med Mol Imaging* **50**, 1090-1102.
- [33] Af Bjerken S, Axelsson J, Larsson A, Flygare C, Remes J, Strandberg S, Eriksson L, Bäckström D, Jakobson Mo S (2023) Reliability and validity of visual analysis of [¹⁸F]FE-PE21 PET/CT in early Parkinsonian disease. *Nucl Med Commun* **44**, 397-406.
- [34] Pineda-Pardo JA, Sánchez-Ferró A, Monje MHG, Pavese N, Obeso JA (2022) Onset pattern of nigrostriatal denervation in early Parkinson's disease. *Brain* **145**, 1018-1028.
- [35] Adams JR, van Netten H, Schulzer M, Mak E, McKenzie J, Strongosky A, Sossi V, Ruth TJ, Lee CS, Farrer M, Gasser T, Uitti RJ, Calne DB, Wszolek ZK, Stoessl AJ (2005) PET in LRRK2 mutations: Comparison to sporadic Parkinson's disease and evidence for presymptomatic compensation. *Brain* **128**, 2777-2785.
- [36] Sossi V, de la Fuente-Fernández R, Nandhagopal R, Schulzer M, McKenzie J, Ruth TJ, Aasly JO, Farrer MJ, Wszolek ZK, Stoessl JA (2010) Dopamine turnover increases in asymptomatic LRRK2 mutations carriers. *Mov Disord* **25**, 2717-2723.
- [37] Chahine LM, Brumm MC, Caspell-Garcia C, Oertel W, Mollenhauer B, Amara A, Fernandez-Arcos A, Tolosa E, Simonet C, Högl B, Videnovic A, Hutten SJ, Tanner C, Weintraub D, Burghardt E, Coffey C, Cho HR, Kiebertz K, Poston KL, Merchant K, Galasko D, Foroud T, Siderowf A, Marek K, Simuni T, Iranzo A (2020) Dopamine transporter imaging predicts clinically-defined α -synucleinopathy in REM sleep behavior disorder. *Ann Clin Transl Neurol* **8**, 201-212.
- [38] Iranzo A, Santamaría J, Valldeoriola F, Serradell M, Salameo M, Gaig C, Niñerola-Baizán A, Sánchez-Valle R, Lladó A, De Marzi R, Stefani A, Seppi K, Pavia J, Högl B, Poewe W, Tolosa E, Lomeña F (2017) Dopamine transporter imaging deficit predicts early transition to synucleinopathy in idiopathic rapid eye movement sleep behavior disorder. *Ann Neurol* **82**, 419-428.
- [39] Chahine LM, Iranzo A, Fernández-Arcos A, Simuni T, Seedorff N, Caspell-Garcia C, Amara AW, Comella C, Högl B, Hamilton J, Marek K, Mayer G, Mollenhauer B, Postuma R, Tolosa E, Trenkwalder C, Videnovic A, Oertel W, PPMI Sleep Working Group (2019) Basic clinical features do not predict dopamine transporter binding in idiopathic REM behavior disorder. *NPJ Parkinsons Dis* **5**, 2.
- [40] Kunz D, Stotz S, de Zeeuw J, Papakonstantinou A, Dümchen S, Haberecht M, Plotkin M, Bes F (2023) Prognostic biomarkers in prodromal α -synucleinopathies: DAT binding and REM sleep without atonia. *J Neurol Neurosurg Psychiatry* **94**, 532-540.
- [41] Knudsen K, Fedorova TD, Horsager J, Andersen KB, Skjærbaek C, Berg D, Schaeffer E, Brooks DJ, Pavese N, Van Den Berge N, Borghammer P (2021) Asymmetric dopaminergic dysfunction in brain-first versus body-first Parkinson's disease subtypes. *J Parkinsons Dis* **11**, 1677-1687.
- [42] Iranzo A, Stefani A, Niñerola-Baizán A, Stokner H, Serradell M, Vilas D, Holzkecht E, Gaig C, Pavia J, Lomeña F, Reyes D, Seppi K, Santamaría J, Högl B, Tolosa E, Poewe W, Sleep Innsbruck Barcelona (SINBAR) Group (2020) Left-hemispheric predominance of nigrostriatal deficit in isolated REM sleep behavior disorder. *Neurology* **94**, e1605-e1613.
- [43] Siderowf A, Jennings D, Stern M, Seibyl J, Eberly S, Oakes D, Marek K, PARS Investigators (2020) Clinical and imaging progression in the PARS cohort: Long-term follow-up. *Mov Disord* **35**, 1550-1557.
- [44] Marrero-González P, Iranzo A, Bedoya D, Serradell M, Niñerola-Baizán A, Perissinotti A, Gaig C, Vilaseca I, Alóbid I, Santamaría J, Molló J (2020) Prodromal Parkinson disease in patients with idiopathic hyposmia. *J Neurol* **267**, 3673-3682.
- [45] Löhle M, Wolz M, Beuthien-Baumann B, Oehme L, van den Hoff J, Kotzerke J, Reichmann H, Storch A (2020) Olfactory dysfunction correlates with putaminal dopamine turnover in early de novo Parkinson's disease. *J Neural Transm (Vienna)* **127**, 9-16.
- [46] Buchert R, Buhmann C, Apostolova I, T. Meyer P, Gallinat J (2019) Nuclear imaging in the diagnosis of clinically uncertain parkinsonian syndromes. *Dtsch Arztebl Int* **116**, 747-754.
- [47] van Eimeren T, Claßen J, Drzezga A, Eggers C, Hilker-Rogendorf R, Klucken J, Koschel J, Meyer PT, Redecker C, Theis H, Buhmann C (2020) [Recommendation for the differentiated use of nuclear medical diagnostic for parkinsonian syndromes]. *Fortschr Neurol Psychiatr* **88**, 609-619.
- [48] Beauchamp LC, Dore V, Villemagne VL, Xu S, Finkelstein D, Barnham KJ, Rowe C (2023) Utilizing 18F-AV-133 VMAT2 PET imaging to monitor progressive nigrostriatal degeneration in Parkinson disease. *Neurology* **101**, e2314-e2324.
- [49] Cilia R, Marotta G, Belletti A, Siri C, Pezzoli G (2014) Reversible dopamine transporter reduction in drug-induced Parkinsonism. *Mov Disord* **29**, 575-577.
- [50] Morbelli S, Esposito G, Arbizu J, Barthel H, Boellaard R, Bohnen NI, Brooks DJ, Darcourt J, Dickson JC, Douglas

- D, Drzezga A, Dubroff J, Ekmekcioglu O, Garibotto V, Herscovitch P, Kuo P, Lammertsma A, Pappata S, Peñuelas I, Seibyl J, Semah F, Tossici-Bolt L, Van de Giessen E, Van Laere K, Varrone A, Wanner M, Zupal G, Law I (2020) EANM practice guideline/SNMMI procedure standard for dopaminergic imaging in Parkinsonian syndromes 1.0. *Eur J Nucl Med Mol Imaging* **47**, 1885-1912.
- [51] Muzio L, Viotti A, Martino G (2021) Microglia in neuroinflammation and neurodegeneration: From understanding to therapy. *Front Neurosci* **15**, 742065.
- [52] Best L, Ghadery C, Pavese N, Tai YF, Strafella AP (2019) New and old TSPO PET radioligands for imaging brain microglial activation in neurodegenerative disease. *Curr Neurol Neurosci Rep* **19**, 24.
- [53] Gerhard A, Pavese N, Hottot G, Turkheimer F, Es M, Hammers A, Eggert K, Oertel W, Banati RB, Brooks DJ (2006) *In vivo* imaging of microglial activation with [¹¹C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis* **21**, 404-412.
- [54] Stokholm MG, Iranzo A, Østergaard K, Serradell M, Otto M, Svendsen KB, Garrido A, Vilas D, Borghammer P, Santamaria J, Møller A, Gaig C, Brooks DJ, Tolosa E, Pavese N (2017) Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: A case-control study. *Lancet Neurol* **16**, 789-796.
- [55] Stokholm MG, Iranzo A, Østergaard K, Serradell M, Otto M, Bacher Svendsen K, Garrido A, Vilas D, Parbo P, Borghammer P, Santamaria J, Møller A, Gaig C, Brooks DJ, Tolosa E, Pavese N (2018) Extrastriatal monoaminergic dysfunction and enhanced microglial activation in idiopathic rapid eye movement sleep behaviour disorder. *Neurobiol Dis* **115**, 9-16.
- [56] Gersel Stokholm M, Garrido A, Tolosa E, Serradell M, Iranzo A, Østergaard K, Borghammer P, Møller A, Parbo P, Staer K, Brooks DJ, Martí MJ, Pavese N (2020) Imaging dopamine function and microglia in asymptomatic LRRK2 mutation carriers. *J Neurol* **267**, 2296-2300.
- [57] Mullin S, Stokholm MG, Hughes D, Mekia A, Parbo P, Hinz R, Pavese N, Brooks DJ, Schapira AHV (2021) Brain microglial activation increased in glucocerebrosidase (GBA) mutation carriers without Parkinson's disease. *Mov Disord* **36**, 774-779.
- [58] Staer K, Iranzo A, Stokholm MG, Østergaard K, Serradell M, Otto M, Svendsen KB, Garrido A, Vilas D, Santamaria J, Møller A, Gaig C, Brooks DJ, Borghammer P, Tolosa E, Pavese N (2020) Cortical cholinergic dysfunction correlates with microglial activation in the substantia innominata in REM sleep behavior disorder. *Parkinsonism Relat Disord* **81**, 89-93.
- [59] Meles SK, Vadasz D, Renken RJ, Sittig-Wiegand E, Mayer G, Depboylu C, Reetz K, Overeem S, Pijpers A, Reesink FE, van Laar T, Heinen L, Teune LK, Höffken H, Luster M, Kesper K, Adriaanse SM, Booi J, Leenders KL, Oertel WH (2017) FDG PET, dopamine transporter SPECT, and olfaction: Combining biomarkers in REM sleep behavior disorder. *Mov Disord* **32**, 1482-1486.
- [60] Orso B, Mattioli P, Yoon E-J, Kim YK, Kim H, Shin JH, Kim R, Liguori C, Famà F, Donniaquio A, Massa F, García DV, Meles SK, Leenders KL, Chiaravalloti A, Pardini M, Bauckneht M, Morbelli S, Nobili F, Lee J-Y, Arnaldi D (2023) Validation of the REM behaviour disorder phenoconversion-related pattern in an independent cohort. *Neurol Sci* **44**, 3161-3168.
- [61] Mattioli P, Orso B, Liguori C, Famà F, Giorgetti L, Donniaquio A, Massa F, Giberti A, Vázquez García D, Meles SK, Leenders KL, Placidi F, Spanetta M, Chiaravalloti A, Camedda R, Schillaci O, Izzi F, Mercuri NB, Pardini M, Bauckneht M, Morbelli S, Nobili F, Arnaldi D (2023) Derivation and validation of a phenoconversion-related pattern in idiopathic rapid eye movement behavior disorder. *Mov Disord* **38**, 57-67.
- [62] Kogan RV, Janzen A, Meles SK, Sittig E, Renken RJ, Gurvits V, Mayer G, Leenders KL, Oertel WH, REM-PET Working Group (2021) Four-year follow-up of [¹⁸F]Fluorodeoxyglucose positron emission tomography-based Parkinson's disease-related pattern expression in 20 patients with isolated rapid eye movement sleep behavior disorder shows prodromal progression. *Mov Disord* **36**, 230-235.
- [63] Carli G, Meles SK, Janzen A, Sittig E, Kogan RV, Perani D, Oertel WH, Leenders KL, REMPET Working Group (2023) Occipital hypometabolism is a risk factor for conversion to Parkinson's disease in isolated REM sleep behaviour disorder. *Eur J Nucl Med Mol Imaging* **50**, 3290-3301.
- [64] Yoon EJ, Lee J-Y, Kim H, Yoo D, Shin JH, Nam H, Jeon B, Kim YK (2022) Brain metabolism related to mild cognitive impairment and phenoconversion in patients with isolated REM sleep behavior disorder. *Neurology* **98**, e2413-e2424.
- [65] Peng S, Tang C, Schindlbeck K, Rydzinski Y, Dhawan V, Spetsieris PG, Ma Y, Eidelberg D (2021) Dynamic [¹⁸F]-FPCIT PET: Quantification of Parkinson's disease metabolic networks and nigrostriatal dopaminergic dysfunction in a single imaging session. *J Nucl Med* **62**, 1775-1782.
- [66] Horsager J, Okkels N, Hansen AK, Damholdt MF, Andersen KH, Fedorova TD, Munk OL, Danielsen EH, Pavese N, Brooks DJ, Borghammer P (2022) Mapping cholinergic synaptic loss in Parkinson's disease: An [¹⁸F]FE0BV PET case-control study. *J Parkinsons Dis* **12**, 2493-2506.
- [67] van der Zee S, Kanel P, Gerritsen MJ, Boertien JM, Slomp AC, Müller MLTM, Bohnen NI, Spikman JM, van Laar T (2022) Altered cholinergic innervation in de novo Parkinson's disease with and without cognitive impairment. *Mov Disord* **37**, 713-723.
- [68] Bohnen NI, Roytman S, Kanel P, Müller MLTM, Scott PJH, Frey KA, Albin RL, Koeppe RA (2022) Progression of regional cortical cholinergic denervation in Parkinson's disease. *Brain Commun* **4**, fca320.
- [69] Slingerland S, van der Zee S, Carli G, Slomp AC, Boertien JM, d'Angremont E, Bohnen NI, Albin RL, van Laar T (2023) Cholinergic innervation topography in GBA-associated de novo Parkinson's disease patients. *Brain*, doi: 10.1093/brain/awad323.
- [70] Staer K, Iranzo A, Terkelsen MH, Stokholm MG, Danielsen EH, Østergaard K, Serradell M, Otto M, Svendsen KB, Garrido A, Vilas D, Santamaria J, Møller A, Gaig C, Brooks DJ, Borghammer P, Tolosa E, Pavese N (2024) Progression of brain cholinergic dysfunction in patients with isolated rapid eye movement sleep behavior disorder. *Eur J Neurol* **31**, e16101.
- [71] Tan C, Nawaz H, Lageman SK, Cloud LJ, Amara AW, Newman BT, Druzgal TJ, Berman BD, Mukhopadhyay N, Barrett MJ (2023) Cholinergic nucleus 4 degeneration and cognitive impairment in isolated rapid eye movement sleep behavior disorder. *Mov Disord* **38**, 474-479.
- [72] de Natale ER, Wilson H, Politis M (2021) Serotonergic imaging in Parkinson's disease. *Prog Brain Res* **261**, 303-338.

- [73] Fazio P, Ferreira D, Svenningsson P, Halldin C, Farde L, Westman E, Varrone A (2020) High-resolution PET imaging reveals subtle impairment of the serotonin transporter in an early non-depressed Parkinson's disease cohort. *Eur J Nucl Med Mol Imaging* **47**, 2407-2416.
- [74] Maillot A, Météreau E, Tremblay L, Favre E, Klinger H, Lhommée E, Le Bars D, Castrioto A, Prange S, Sgambato V, Broussolle E, Krack P, Thobois S (2021) Serotonergic and dopaminergic lesions underlying parkinsonian neuropsychiatric signs. *Mov Disord* **36**, 2888-2900.
- [75] Pavese N, Metta V, Bose SK, Chaudhuri KR, Brooks DJ (2010) Fatigue in Parkinson's disease is linked to striatal and limbic serotonergic dysfunction. *Brain* **133**, 3434-3443.
- [76] Barber TR, Griffanti L, Muhammed K, Drew DS, Bradley KM, McGowan DR, Crabbe M, Lo C, Mackay CE, Husain M, Hu MT, Klein JC (2018) Apathy in rapid eye movement sleep behaviour disorder is associated with serotonin depletion in the dorsal raphe nucleus. *Brain* **141**, 2848-2854.
- [77] Doppler CEJ, Kinnerup MB, Brune C, Farrher E, Betts M, Fedorova TD, Schaldemose JL, Knudsen K, Ismail R, Seger AD, Hansen AK, Stær K, Fink GR, Brooks DJ, Nahimi A, Borghammer P, Sommerauer M (2021) Regional locus coeruleus degeneration is uncoupled from noradrenergic terminal loss in Parkinson's disease. *Brain* **144**, 2732-2744.
- [78] Laurencin C, Lancelot S, Brosse S, Mérida I, Redouté J, Greusard E, Lamberet L, Liotier V, Le Bars D, Costes N, Thobois S, Boulinguez P, Ballanger B (2023) Noradrenergic alterations in Parkinson's disease: A combined 11C-yohimbine PET/neuromelanin MRI study. *Brain*, doi: 10.1093/brain/awad338.
- [79] Knudsen K, Fedorova TD, Hansen AK, Sommerauer M, Otto M, Svendsen KB, Nahimi A, Stokholm MG, Pavese N, Beier CP, Brooks DJ, Borghammer P (2018) *In-vivo* staging of pathology in REM sleep behaviour disorder: A multimodality imaging case-control study. *Lancet Neurol* **17**, 618-628.
- [80] Brumberg J, Kuzkina A, Lapa C, Mammadova S, Buck A, Volkmann J, Sommer C, Isaacs HJ, Doppler K (2021) Dermal and cardiac autonomic fiber involvement in Parkinson's disease and multiple system atrophy. *Neurobiol Dis* **153**, 105332.
- [81] Hawkes CH, Del Tredici K, Braak H (2007) Parkinson's disease: A dual-hit hypothesis. *Neuropathol Appl Neurobiol* **33**, 599-614.
- [82] Knudsen K, Fedorova TD, Hansen AK, Sommerauer M, Haase A-M, Svendsen KB, Otto M, Østergaard K, Krogh K, Borghammer P (2019) Objective intestinal function in patients with idiopathic REM sleep behavior disorder. *Parkinsonism Relat Disord* **58**, 28-34.
- [83] Siebner TH, Fuglsang S, Madelung CF, Løkkegaard A, Bendtsen F, Hove JD, Damgaard M, Madsen JL, Siebner HR (2022) Gastric emptying is not delayed and does not correlate with attenuated postprandial blood flow increase in medicated patients with early Parkinson's disease. *Front Neurol* **13**, 828069.
- [84] Horsager J, Andersen KB, Knudsen K, Skjærbæk C, Fedorova TD, Okkels N, Schaeffer E, Bonkat SK, Geday J, Otto M, Sommerauer M, Danielsen EH, Bech E, Kraft J, Munk OL, Hansen SD, Pavese N, Göder R, Brooks DJ, Berg D, Borghammer P (2020) Brain-first versus body-first Parkinson's disease: A multimodal imaging case-control study. *Brain* **143**, 3077-3088.
- [85] Totsune T, Baba T, Sugimura Y, Oizumi H, Tanaka H, Takahashi T, Yoshioka M, Nagamatsu K-I, Takeda A (2023) Nuclear imaging data-driven classification of Parkinson's disease. *Mov Disord* **38**, 2053-2063.
- [86] Fearon C, Lang AE, Espay AJ (2021) The logic and pitfalls of Parkinson's disease as "brain-first" versus "body-first" subtypes. *Mov Disord* **36**, 594-598.
- [87] Kim MS, Park DG, An Y-S, Yoon JH (2023) Dual-phase 18 F-FP-CIT positron emission tomography and cardiac 123 I-MIBG scintigraphy of Parkinson's disease patients with GBA mutations: Evidence of the body-first type? *Eur J Neurol* **30**, 344-352.
- [88] Park DG, Kang J, An Y-S, Chang J, Yoon JH (2022) Association of plasma α -synuclein with cardiac 123I-MIBG scintigraphy in early Parkinson's disease. *Neurosci Lett* **770**, 136399.
- [89] Pitton Rissardo J, Fornari Caprara AL (2023) Cardiac 123I-metaiodobenzylguanidine (MIBG) scintigraphy in Parkinson's disease: A comprehensive review. *Brain Sci* **13**, 1471.
- [90] Bombardieri E, Gianmarile F, Aktolun C, Baum RP, Bischof Delaloye A, Maffioli L, Moncayo R, Mortelmans L, Pepe G, Reske SN, Castellani MR, Chiti A, European Association for Nuclear Medicine (2010) 134I/123I metaiodobenzylguanidine (mIBG) scintigraphy: Procedure guidelines for tumour imaging. *Eur J Nucl Med Mol Imaging* **37**, 2436-2446.
- [91] Stuparu AZ, Jurja S, Stuparu AF, Axelerad A (2023) Narrative review concerning the clinical spectrum of ophthalmological impairments in Parkinson's disease. *Neurol Int* **15**, 140-161.
- [92] Mailankody P, Lenka A, Pal PK (2019) The role of optical coherence tomography in Parkinsonism: A critical review. *J Neurol Sci* **403**, 67-74.
- [93] Deng Y, Jie C, Wang J, Liu Z, Li Y, Hou X (2022) Evaluation of retina and microvascular changes in the patient with Parkinson's disease: A systematic review and meta-analysis. *Front Med (Lausanne)* **9**, 957700.
- [94] Rascunà C, Cicero CE, Chisari CG, Russo A, Giuliano L, Castellino N, Terravecchia C, Grillo M, Longo A, Avitabile T, Zappia M, Reibaldi M, Nicoletti A (2021) Retinal thickness and microvascular pathway in Idiopathic Rapid eye movement sleep behaviour disorder and Parkinson's disease. *Parkinsonism Relat Disord* **88**, 40-45.
- [95] Cerveró A, Sánchez-Rodríguez A, Rivera-Sánchez M, Martínez-Rodríguez I, Sierra M, González-Aramburu I, Gutiérrez-González A, Andrés-Pacheco J, Sánchez-Peláez MV, Casado A, Infante J (2023) Analysis of retinal nerve layers in idiopathic, LRRK2-associated Parkinson's disease and unaffected carriers of G2019S mutation. *Parkinsonism Relat Disord* **106**, 105246.
- [96] Bischof GN, Ewers M, Franzmeier N, Grothe MJ, Hoenig M, Kocagoncu E, Neitzel J, Rowe JB, Strafella A, Drzezga A, van Eimeren T, MINC faculty (2019) Connectomics and molecular imaging in neurodegeneration. *Eur J Nucl Med Mol Imaging* **46**, 2819-2830.
- [97] Chougar L, Arsovic E, Gaurav R, Biondetti E, Faucher A, Valabrègue R, Pyatigorskaya N, Dupont G, Lejeune F-X, Cormier F, Corvol J-C, Vidailhet M, Degos B, Grabli D, Lehericy S (2022) Regional selectivity of neuromelanin changes in the substantia nigra in atypical parkinsonism. *Mov Disord* **37**, 1245-1255.
- [98] Fan Z, Aman Y, Ahmed I, Chetelat G, Landeau B, Ray Chaudhuri K, Brooks DJ, Edison P (2015) Influence of microglial activation on neuronal function in Alzheimer's

- and Parkinson's disease dementia. *Alzheimers Dement* **11**, 608-621.e7.
- [99] Janzen A, Vadasz D, Booij J, Luster M, Librizzi D, Henrich MT, Timmermann L, Habibi M, Sittig E, Mayer G, Geibl F, Oertel W (2022) Progressive olfactory impairment and cardiac sympathetic denervation in REM sleep behavior disorder. *J Parkinsons Dis* **12**, 1921-1935.
- [100] Smith R, Capotosti F, Schain M, Ohlsson T, Vokali E, Molette J, Touilloux T, Hliva V, Dimitrakopoulos IK, Puschmann A, Jögi J, Svenningsson P, Andréasson M, Sandiego C, Russell DS, Miranda-Azpiazu P, Halldin C, Stomrud E, Hall S, Bratteby K, Tampio L'Estrade E, Luthi-Carter R, Pfeifer A, Kosco-Vilbois M, Streffer J, Hansson O (2023) The α -synuclein PET tracer [18F] ACI-12589 distinguishes multiple system atrophy from other neurodegenerative diseases. *Nat Commun* **14**, 6750.
- [101] Fernandes Gomes B, Farris CM, Ma Y, Concha-Marambio L, Lebovitz R, Nellgård B, Dalla K, Constantinescu J, Constantinescu R, Gobom J, Andreasson U, Zetterberg H, Blennow K (2023) α -Synuclein seed amplification assay as a diagnostic tool for parkinsonian disorders. *Parkinsonism Relat Disord* **117**, 105807.

CORRECTED PROOF