

Cite this article as: Neural Regen Res. 2012;7(8):611-619.

Magnetic resonance imaging markers for early diagnosis of Parkinson's disease[☆]

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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder characterized by selective and progressive degeneration, as well as loss of dopaminergic neurons in the substantia nigra. In PD, approximately 60–70% of nigrostriatal neurons are degenerated and 80% of content of the striatal dopamine is reduced before the diagnosis can be established according to widely accepted clinical diagnostic criteria. This condition describes a stage of disease called "prodromal", where non-motor symptoms, such as olfactory dysfunction, constipation, rapid eye movement behaviour disorder, depression, precede motor sign of PD. Detection of prodromal phase of PD is becoming an important goal for determining the prognosis and choosing a suitable treatment strategy. In this review, we present some non-invasive instrumental approaches that could be useful to identify patients in the prodromal phase of PD or in an early clinical phase, when the first motor symptoms begin to be apparent. Conventional magnetic resonance imaging (MRI) and advanced MRI techniques, such as magnetic resonance spectroscopy imaging, diffusion-weighted and diffusion tensor imaging and functional MRI, are useful to differentiate early PD with initial motor symptoms from atypical parkinsonian disorders, thus, making easier early diagnosis. Functional MRI and diffusion tensor imaging techniques can show abnormalities in the olfactory system in prodromal PD.

Key Words: Parkinson's disease; early diagnosis; conventional magnetic resonance imaging; magnetic resonance spectroscopy; diffusion-weighted imaging; diffusion tensor imaging; functional magnetic resonance imaging; olfactory dysfunction

Abbreviations: PD, Parkinson's disease; SN, substantia nigra; OB, olfactory bulb; PSP, progressive supranuclear palsy; MSA, multiple-system atrophy; CBD, corticobasal degeneration.

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Received: 2011-11-26
Accepted: 2012-02-02
(NY20111126001/ZLJ)

Marino S, Ciurleo R, Di
Lorenzo G, Barresi M, De
Salvo S, Giacoppo S,
Bramanti A, Lanzafame P,
Bramanti P. Magnetic
resonance imaging markers
for early diagnosis of
Parkinson's disease. Neural
Regen Res.
2012;7(8):611-619.

www.crter.cn
www.nrronline.org

doi:10.3969/j.issn.1673-5374.
2012.08.009

INTRODUCTION

Parkinson's disease (PD) is a progressive disorder with a relentless neuronal cell loss in several brain areas and nuclei notably in the substantia nigra (SN). The course of this neuronal loss is still unclear and may be highly variable in different PD patients and in different phases of the disease. At present, no treatment has proven to influence this progressive course of the disease by protecting neurons or by postponing cell death.

One potential reason for the lack of neuroprotective effects of various agents, which have been highly effective in animal experiments, is the fact that the neurodegenerative process has already substantially proceeded when the diagnosis is established on the basis of widely accepted diagnostic criteria for PD: when the patients fulfill the clinical criteria of PD, 60–70% of neurons of the SN are degenerated and the striatal dopamine content is reduced by 80%, suggesting that the remaining neurons of the

SN are also altered^[1].

The "preclinical" phase may give the incorrect impression of patients exhibiting no clinical signs or symptoms of the incipient disease. Conversely, it is known that motor signs develop insidiously and minor signs of asymmetric hypokinesia may be detected years before the diagnosis of PD can be established. In addition, non-motor symptoms such as mood disorders, olfactory, vegetative, sensory or neuropsychological signs may be noticed by the patients or physicians in advance of motor signs reflecting the dysfunction of dopaminergic or non-dopaminergic neurons. Therefore, the term "prodromal" phase of PD would more appropriately characterize this stage of the disease. The clinical impression of autonomic, olfactory and affective symptoms preceding motor signs of PD are in line with the findings demonstrating that neuronal alteration, with regard to Lewy body formation, occurs first in the dorsal vagal nucleus, the olfactory bulb (OB), the raphe and coeruleus nuclei before entering the SN^[2-3].

According to neuropathological findings, it is suggested that approximately 10% of subjects older than 60 years are in the prodromal phase of PD. These subjects exhibit the pathological hallmarks of PD, like Lewy bodies and neuronal loss at the SN, without showing the motor signs during life time that allow the diagnosis of PD. In only 10% of this group with so-called "incidental Lewy body disease", neuronal loss will proceed reaching the degree where motor symptoms are distinct enough to allow the diagnosis of PD^[4].

It would be of great interest with respect to research and treatment to identify those subjects at risk 1) to initiate neuroprotective treatment earlier and 2) to define the causes of more rapid neuronal loss and disease progression in those patients with "incidental Lewy body disease" who will cross the threshold of critical neuronal loss at the SN and develop PD.

The duration of the prodromal period remains unknown. The duration of this phase of PD was estimated to last from a few years up to several decades before the first symptoms are noticed by the patients^[1].

Several procedures have been proposed to identify subjects in prodromal stages of PD. In this review, we present some instrumental approaches to identify markers in prodromal phase of PD or in an early clinical phase, when the first motor symptoms begin to be apparent. These instrumental approaches could be useful to help the clinicians in early diagnosis.

MAGNETIC RESONANCE IMAGING (MRI) IN DIAGNOSIS OF PARKINSON'S DISEASE

PD in early stages of motor symptoms can easily be mistaken for any number of disorders. Indeed PD is most likely to be confused with various atypical parkinsonian disorders (APDs), such as progressive supranuclear palsy (PSP), multiple-system atrophy (MSA), especially the Parkinson variant of multiple-system atrophy (MSA-P), and corticobasal degeneration (CBD).

A differentiation of these clinical entities may be challenging, particularly in the early stages of motor symptoms of the disease, where overlapping clinical signs lead to a high rate of misclassification. However, a differentiation between APDs and PD is important for making easier early diagnosis and choosing a specific treatment strategy.

MRI plays an important role in the differential diagnosis in PD. Conventional MRI (cMRI), as well as different advanced MRI techniques, including magnetic resonance spectroscopy (MRS), diffusion-weighted and diffusion tensor imaging (DWI/DTI) and functional MRI (fMRI), are helpful to distinguish PD from APDs, especially in early stage of disease where a differentiation of these conditions is not easy (Table 1).

Table 1 Major results for MRI techniques

MRI technique	Disease	Result	Reference
cMRI	PD	Increase of signal in T2-weighted MRI, smudging of the hypointensity, and signal loss in inversion recovery MRI in SN.	5-7
¹ HMRS	PD	Shortening of T2 values in SN, caudate and putamen.	8
	MSA-P	Reduction of NAA/Cr ratio in the lentiform nucleus.	9
	PSP and CBD	Reduction of NAA/Cr ratio in the frontal cortex and putamen.	10
	PD	Normal level of NAA/Cr ratio in the frontal cortex and putamen.	10
	MSA-P and PSP	Reduction of NAA levels in the pallidum, putamen, and lentiform nucleus.	11
	MSA and PSP	Reduction of NAA/Cho levels.	12
¹ HMRS at 3T	MSA, PSP, and PD	Reduction of NAA/Cr levels.	12
	APD and PD	Reduction of NAA/Cho and NAA/Cr levels.	13-14
DWI	MSA-C and MSA-P	Reduction of NAA/Cr levels in pontine basis.	15
	MSA-P	Reduction of NAA/Cr levels in putamen.	15
DTI	PSP	High value of apparent diffusion coefficient in superior peduncle.	16
	PD	Normal value of apparent diffusion coefficient in superior peduncle.	16
DTI at 3T	De novo PD	Increase of fractional anisotropy histogram.	17
	akinetic-rigid type		
fMRI	De novo PD	Reduction of fractional anisotropy value in the caudal regions of interest of the SN, frontal lobes, SMA, pre-SMA, and cingulum.	18
	PD	Reduction of cerebral activation in recognition memory networks.	19
rsfcMRI	PD	Increase of FC in the primary motor cortex and the cerebellum. Reduction of FC in the MSA, the dorsolateral prefrontal areas, and the putamen.	20
		Increase of FC between the pre-SMA and the right primary motor cortex and decrease of FC between the pre-SMA and the left putamen.	21
		Decrease of FC between the posterior putamen and the inferior parietal cortex.	22
	Early PD	Increase of FC between the subthalamic nucleus and the cortical motor areas.	23

MRI: Magnetic resonance imaging; cMRI: conventional MRI; PD: Parkinson's disease; SN: substantia nigra; ¹HMRS: proton magnetic resonance spectroscopy; MSA: multiple-system atrophy; MSA-P: Parkinson variant of multiple-system atrophy; PSP: progressive supranuclear palsy; CBD: corticobasal degeneration; DWI: diffusion-weighted imaging; DTI: diffusion tensor imaging; fMRI: functional MRI; rsfcMRI: resting state functional connectivity MRI; SMA: supplementary motor area; NAA: N-acetylaspartate; Cr: creatine + phosphocreatine; Cho: choline-containing compounds; FC: functional connectivity.

cMRI

The principles of MRI are based on the ubiquitous presence of hydrogen in body tissues and the spin of the hydrogen atom proton, which induces a small magnetic field. In general, T2-weighted sequences are sensitive to changes in tissue properties, including tissue damage, due to changes of the transverse magnetization or T2 decay. Neurodegenerative processes are characterized by cell loss, increased age-related deposition of iron or other paramagnetic substances, and by astroglial reaction and microglial proliferation may lead to signal changes in affected brain areas, like the basal ganglia or infratentorial structures, in neurodegenerative parkinsonism^[24-27].

Because cMRI is believed to be usually normal in patients with PD, while it frequently shows characteristic abnormalities in patients with APDs, cMRI images takes a major part in excluding underlying pathologies such as vascular lesions, multiple sclerosis, brain tumors, normal pressure hydrocephalus, bilateral striopallidodentate calcinosis, and other potential, but rare causes of symptomatic parkinsonism such as Wilson disease, manganese-induced parkinsonism, or different subtypes of neurodegeneration associated with brain iron accumulation.

At 1.5 Tesla, patients with advanced PD, and sometimes those with APDs, may show distinct abnormalities of the SN, including signal increase on T2-weighted MR images, smudging of the hypointensity in the SN towards the red nucleus or signal loss when using inversion recovery MR^[5-7].

Biochemical studies have reported increased iron content in the SN pars compacta in PD, with changes most marked in severe disease, suggesting that measurement of nigral iron content may provide an indication of severity of the disease^[28]. Iron accumulates in the brain as a function of age, primarily in the form of ferritin and particularly in oligodendrocytes, but also in neurons and microglia. The adult brain has a very high iron content, particularly in the basal ganglia. Brain iron concentration is highest in the globus pallidus, SN, red nucleus, caudate, and putamen. Abnormally elevated iron levels are evident in various neurodegenerative disorders, including PD where there is evidence of increased iron in the SN^[29-30]. Signal changes on T2-weighted images in the basal ganglia as well as in infratentorial structures have been reported for all APDs at 1.5 Tesla, where they have been used as a differentiating criterion from PD. Furthermore, estimation of transverse relaxation in patients with PD, using a 1.5 Tesla whole body imaging system, showed shortened T2 values in SN, caudate and putamen in PD patients as compared to healthy controls^[8]. These data do suggest a potential utility of these measurements as a biomarker of disease progression.

MRS imaging

MRS is a non-invasive imaging technique that enables *in vivo* quantification of certain neurochemical compounds.

MRS has been demonstrated *in vivo* for different nuclei, including ¹H, ³¹P, ¹³C, ¹⁵N, ¹⁹F and ²³Na. The main nucleus studied today in neurospectroscopy is ¹H which provides information on markers of neurons, myelin, energy metabolism and other metabolically active compounds.

The metabolites detectable with proton MRS (¹HMRS) include the prominent resonances of *N*-acetylaspartate (NAA), choline-containing compounds (Cho), creatine + phosphocreatine (Cr), myo-inositol (mI), lactate (Lac), and a variety of other resonances that might not be evident depending on type and quality of spectra as well as on the pathological condition^[31].

NAA is widely interpreted as a neuronal marker and implicated in several neuronal processes, including lipid and protein synthesis, mitochondrial functioning and osmoregulation. NAA is reduced in many brain disorders in presence of neuronal or axona loss. The Cho peak represents a combination of several choline-containing compounds, including free Cho, phosphorylcholine and glycerophosphorylcholine, and to a small extent acetylcholine. Free Cho acts as a precursor to acetylcholine, while glycerophosphorylcholine is a product of breakdown of membrane phosphatidylcholine and acts as an osmoregulator. The Cho peak is often viewed as a marker of membrane turnover or inflammation in ¹HMRS studies. The Cr peak is composed of creatine and phosphocreatine. These metabolites buffer the energy use and energy storage of cells. Cr concentration is often used as an internal standard because it is considered to be relatively stable, showing slow increase with age. The mI peak represents the presence of myo-inositol and myo-inositol phosphate. MI is suggested as a glial marker, osmoregulator, intracellular messenger and detoxifying agent. The Lac is an end product of anaerobic glycolysis, thus increase in Lac concentrations often serves as an index of altered oxidative metabolism, e.g. in ischemia, hypoxia, and cancer^[31].

MRS is implemented as single-voxel and multi-voxels methods. Single-voxel spectroscopy detects the signal from a single region during one measurement, whereas multi-voxel or MR spectroscopic imaging or chemical shift imaging, using additional phase-encoding pulses, obtains the signal from multiple regions at the same time and provides the information of spatial distribution of major cerebral metabolites.

The concentration changes of all metabolites detected by ¹HMRS could help to evaluate PD subjects with early motor symptoms, especially in early differential diagnosis.

¹HMRS of striatal structures might differentiate PD from APDs by virtue of reduced NAA/Cr ratios in MSA but not PD. In particular ¹HMRS showed reduced NAA/Cr ratios in the lentiform nucleus in six of seven MSA-P cases, whereas normal levels of putaminal NAA were found in eight of nine PD subjects^[9].

As compared to normal controls, in patients with PSP,

CBD, and MSA, but not in those with PD, significant reduction of the NAA/Cr ratio in the frontal cortex was found^[10]. Patients with CBD showed significant reduction of the NAA/Cr ratio in the frontal cortex and putamen as compared to patients with PD and MSA. Patients with PSP showed a significant reduction of the NAA/Cr ratio in the putamen as compared with patients with PD and MSA. Patients with CBD showed clear asymmetry in the putamen as compared to controls and other patients. Guevara *et al*^[11] found that patients with PSP and MSA-P had lower NAA concentrations in the pallidum, putamen and lentiform nucleus compared to healthy controls and patients with PD.

In another study Federico *et al.* showed that NAA/Cho peak ratio was significantly reduced in MSA and in PSP patients compared to PD patients and to control. Moreover the NAA/Cr peak ratio was significantly reduced in MSA, in PSP and in PD patients also compared to controls, but only in MSA compared to PD patients^[12].

However, other MRS studies have shown reduced NAA/Cr and NAA/Cho ratios in the lentiform nucleus not only in APD, but also in PD^[13-14].

Technical factors such as magnetic resonance spectroscopy technique including different echo- and relaxation times, voxel sizes, field strength and pulse sequences used in the different studies, may be responsible for some of the variation of results seen in the published literature on ¹HMRS for the differential diagnosis of neurodegenerative parkinsonism. The development of ¹HMRS at higher magnetic field strengths may lead ¹HMRS to a more important role as imaging tool in the differential diagnosis of parkinsonian disorders.

¹HMRS of the brain with high magnetic field at 3 Tesla has many advantages that, with respect to the well-established and technologically advanced 1.5 Tesla ¹HMRS, include better signal-noise ratio and increased spectral, spatial and temporal resolution, allowing a more reliable estimation of peak area and hence a more precise quantification^[32].

This has been shown by a recent study applying multiple regional single voxel ¹HMRS including putamen, pontine basis and cerebral white matter (WM) at 3 Tesla in 24 patients with MSA compared to 11 PD patients and 18 controls. Significant NAA/Cr reductions have been shown in the pontine basis of both patients with MSA-C (cerebellar ataxia variant of MSA) and MSA-P, while putaminal NAA/Cr was only reduced in the patients with MSA-P. Eight of the 11 MSA-P patients compared to none of the PD and control group were classified correctly by combining individual NAA/Cr reductions in the pontine basis and in the putamen. These results suggest that combined assessment of NAA/Cr in the pontine basis and putamen by higher magnetic field may be effective in differentiating MSA-P from PD in terms of the high specificity of reduced NAA/Cr in the pontine basis or in the putamen in patients with MSA-P^[15].

However in all the cited studies the metabolite concentrations were expressed in terms of semiquantitative ratios such as NAA/Cr, NAA/Cho, Cho/Cr and ml/Cr. In relative quantification, one of the metabolite peaks measured is used as the concentration standard and serves as the denominator of the peak ratios. The assumption that the concentration of certain reference metabolites (e.g. total creatine, choline) remains constant may be incorrect under normal conditions, as well as in many pathologic states. It is therefore advisable to obtain concentration expressed in standard units (such as millimoles per kilogram wet weight) by applying absolute quantification^[33].

In vivo MRS is increasingly utilized for the study of neurochemistry and cerebral energy metabolism in PD. Particularly, the recent technical advances of *in vivo* MRS including the availability of higher magnetic fields permitting improved spectral and spatial resolution, the development of a reliable method for absolute metabolite quantification, and the development of various spectroscopic methods to enhance metabolite signal identification can be a great use to examine the changes in neurochemical profile non-invasively and to achieve a differential diagnosis of PD versus other forms of parkinsonism, especially in early stages of disease when signs and symptoms of different forms of parkinsonism have greater overlap. However, several multicentre trials using a larger sample of patients, absolute quantification of tissue metabolite concentrations and a standardized technique are required to fully determine the place of MRS in early clinical differential diagnosis.

Diffusion-weighted imaging (DWI)

DWI visualizes the random movement of water molecules in the tissue by applying diffusion-sensitizing gradients to assess changes in diffusion magnitude and orientation of water molecules in tissue. Quantification of the diffusivity is achieved by applying diffusion-sensitizing gradients of different degrees in 3 orthogonal directions and calculating the apparent diffusion coefficient (ADC) for each direction. The ADC is very dependent on the direction of diffusion encoding. The random translational motion (diffusion) of water molecules in tissue is restricted by the highly organized architecture of fiber tracts in the central nervous system. Neuronal loss and gliosis disrupt this architecture, resulting in an increase of diffusivity and ADC. The complex neuronal architecture with its organization in fiber bundles that are surrounded by dense myelin sheaths leads also to a distinct anisotropy of water diffusion, which is facilitated along the direction of fiber tracts and restricted perpendicular to the fibers. DWI and the calculation of the ADC have been applied in the diagnostic evaluation of neurodegenerative diseases where modification of the microstructural integrity of nervous tissue leads to an increase of ADC values. In particular, calculation of the ADC allowed to differentiate atypical Parkinsonism from PD supporting so the differential diagnosis. Indeed, the higher values of ADC

in superior cerebellar peduncle of patients with PSP than patients with PD was found^[16].

DTI

DTI is a novel MRI technique that enables non-invasive *in vivo* visualization of brain white-matter microstructure. The degree of anisotropy can be quantified by applying diffusion-sensitizing gradients in at least six directions, which permits calculation of fractional anisotropy (FA). Decreased FA values represent tissue degeneration due to normal aging or due to pathologic reasons such as neurodegeneration. Both diffusivity and FA can be combined to form the so-called diffusion tensor, which indicates direction and extent of diffusivity with the help of a vector^[34-36]. The central nervous system is highly organized in numerous tracts of myelinated fibre bundles, whereby the movement of the water molecules is restricted perpendicular to these fibre bundles. The resulting anisotropic diffusion is quantified by the FA, which is determined by diffusion-sensitized gradients in at least six directions. Both the diffusivity and the FA form the diffusion tensor^[34].

Widespread cerebral changes are observed in advanced stages of PD, suggesting that PD is a multisystem disorder.

Recently, several studies pointed out the capability of the histogram analysis of the apparent diffusion coefficient computed from DWI and of the mean diffusivity and FA computed from DTI to reveal brain-tissue damage in early clinical stages of neurodegenerative diseases. A recent study including 27 patients with de novo drug-naïve PD hypothesized that global measurements of brain volume and structure, such those possible with SIENAX software (part of FSL 4.0

<http://www.fmrib.ox.ac.uk/fsl/>) and histogram analysis of DTI could reveal subtle tissue changes in the early clinical phase of PD^[17]. Accordingly, were investigated with SIENAX and DTI a group of patients with drug-naïve de novo PD and a group of 16 healthy controls. Results showed no significant differences for total brain, gray matter, and WM volumes and histogram-derived mean diffusivity metrics between controls and the whole group of patients with PD or any subgroup of patients with PD. As compared with controls, patients with PD as a whole and patients with the akinetic-rigid type showed an increase of the twenty-fifth percentile of the FA histogram. In patients with the akinetic-rigid type, there also was a trend toward an increase of fiftieth and seventy-fifth percentiles, and a reduction of the skewness of the fractional anisotropy histogram. This finding is consistent with the hypothesis that subtle gray matter loss is present in patients with PD since the early clinical phases and that this feature is more pronounced in patients with akinetic-rigid type. Another recent study including only patients with newly diagnosed PD used high-resolution DTI at 3 T to evaluate rostral, middle, and caudal regions of interest (ROIs) within the SN on a single slice of the midbrain and this study found that PD patients could be completely separated from the control group based on

reduced FA values in the caudal ROI of the SN, such that further confirmatory studies seem warranted. By using statistical parametric mapping analysis of DTI, changes in FA were found in the frontal lobes, including the supplementary motor area (SMA), the pre-SMA, and the cingulum in non demented PD patients relative to controls, whereas voxel-based morphometry analysis in the same patients revealed no volume loss^[18]. These results confirm that the neurodegenerative process extends beyond the basal ganglia in PD^[17]. Concluding DWI and DTI especially bears several advantages. These techniques may detect diffusion abnormalities in the basal ganglia and infratentorial structures in patients with PD at an early stage of disease.

fMRI

fMRI is imaging method based on increased blood flow into local vasculature that accompanies neuronal activation in different brain's areas. This result in a corresponding local reduction in deoxyhemoglobin because the increase in blood flow occurs without an increase of similar magnitude in oxygen extraction^[37]. Functional activity of the brain determined from the magnetic resonance signal has confirmed known anatomically distinct processing areas in the visual cortex^[38], the motor cortex, and Broca's area of speech and language-related activities^[39]. Further a rapidly emerging body of literature documents corresponding findings between fMRI and conventional electrophysiological techniques to localize specific functions of the human brain^[40-41]. Consequently, the number of medical and research centers with fMRI capabilities and investigational programs continue to escalate. The main advantages to fMRI as a technique to image brain activity related to a specific task or sensory process include: 1) the signal does not require injections of radioactive isotopes; and 2) the in-plane resolution of the functional image is generally about 1.5 x 1.5 mm although resolutions less than 1 mm are possible. fMRI has been extensively used to explore in many diseases, like PD, the functional neuroanatomy of cognitive functions.

At the moment only few fMRI studies was performed in early PD, and all have mainly focused on the assessment of brain activity related to impaired cognitive functions.

A recent study has showed that in PD fMRI can detect early dysfunctions in the recognition memory network prior to a clear clinical evidence of a recognition memory deficit^[42].

In this study, the recognition memory task, consisting in a list 35 words, in both PD and healthy controls activated the dorsolateral orbito-frontal cortex and frontal lobe. Other areas have been consistently associated with recognition memory in healthy subjects such as cingulate and paracingulate, and the occipital and cerebellar regions^[19]. These areas showed activation in both PD and control subjects during the performance of the

recognition memory task. Furthermore, a reliable pattern of activity in the medial temporal lobe can be identified across fMRI studies of recognition memory^[43]. This pattern is consistent with a model suggesting that whereas the hippocampus and the parahippocampal gyrus play a specific role for recollection, the perirhinal cortex contributes to familiarity-based recognition^[44]. However, in this study, the hippocampus showed a pattern of deactivation in both patients and controls while performing the recognition memory task probably reflecting the involvement of the hippocampus in the default mode network. Indeed, task-related deactivations for both patients and controls were found in precuneus cortex, middle temporal gyrus, ventro-medial prefrontal cortex, temporal pole and medial temporal regions. The comparison between PD and healthy subjects showed that, even though PD patients presented task-related activations in recognition memory networks, these activations were significantly decreased in the PD group compared to controls.

Previous fMRI studies have found abnormal fronto-striatal activity in early PD. Studies have also suggested that there is relation between dopamine concentrations in the prefrontal cortex and neural function, with different optimal dopaminergic states for motor and cognitive functions^[45-46]. fMRI shows limitations because do not are fully known the pathological basis of PD. Therefore, fMRI remains an indirect measure of disease progression in PD. However, studies of functional connectivity of the motor network may play an important role in understanding functional changes because multiple areas are likely to be involved in the control of a given motor task. Resting state functional connectivity MRI (rsfMRI) is a novel technique which allows the investigation of large scale functional networks at whole brain level based on the temporal correlation of spontaneous, *i.e.* non task-related, blood oxygen level dependent fluctuations in a very low frequency range^[47]. To date, only few studies have applied this technique to investigate network characteristics in PD^[20-22]. Using a global functional connectivity (FC) measure, Wu *et al*^[20] found an increased overall network coupling in the primary motor cortex and the cerebellum, whereas global FC was reduced in the SMA, in dorsolateral prefrontal areas and in the putamen. Recently, an increased FC between the pre-SMA and the right primary motor cortex and a decreased FC between the pre-SMA and the left putamen were reported by these authors^[21]. In addition, Helmich *et al*^[22] found a FC decrease in a network linking the posterior putamen with the inferior parietal cortex arguing for disturbed sensorimotor integration in PD. However, these previous studies did not explicitly focus on the subthalamic nucleus which likely plays a crucial role in normal motor functioning and in the pathophysiology of PD. Baudrexel *et al*^[23] have studied alterations in the FC profile of the subthalamic nucleus. The analysis revealed increased FC between the

subthalamic nucleus and cortical motor areas (BA 4 and 6) in PD patients with early motor symptoms. In conclusion, investigation of the connectivity of brain networks motor-related areas may provide insights to our understanding on abnormality of baseline state brain function in PD responsible for atypical motor functioning and to have an early picture of pathophysiological state of the disease.

MRI EVALUATION OF OLFACTORY DYSFUNCTION IN PD: AN EARLY DIAGNOSTIC MARKER

Olfactory dysfunction is a frequent non-motor symptom in PD and may be considered as an early clinical feature of the disease preceding motor symptoms by year^[48]. More than 96% of patients with PD have olfactory dysfunction, compared with an established olfactory loss of at least 25% in the normal population over 52 years of age^[49].

The majority of PD patients are functionally anosmic or severely hyposmic. The cause of hyposmia in PD is not yet fully understood. It has been proposed that the development of Lewy inclusion bodies, starting from the medulla oblongata and the anterior olfactory nucleus before the involvement of other central nervous structures, constitutes the reason of olfactory impairment before the motor symptoms appearance^[50]. Moreover olfactory loss in PD is not a primary consequence of damage to the olfactory epithelium but rather result from distinct central nervous system abnormalities^[51]. Studies based on biopsies from the olfactory epithelium did not reveal specific changes in the nasal mucosa of PD patients compared to patients who were hyposmic for other reasons (rhinitis, smoking or toxic agents). With regard to volumetrics of the olfactory bulb (OB) results indicated that there is little or no difference between PD patients with anosmia/hyposmia and healthy normosmic controls in terms of OB volume^[52]. Support for these results has come from a study that found an increase of (inhibitory) dopaminergic neurons in the OB in PD patients^[53]. These findings have been interpreted within the context of a possible compensatory mechanism in response to the loss of dopaminergic neurons in the basal ganglia. While cardinal motor symptoms in PD are closely related to a severe loss of dopaminergic cells in the nigro-striatal pathway, early clinical features such as olfactory impairment are more likely to be associated with extranigral pathology. Indeed atrophy in olfactory regions of the limbic and paralimbic cortex in early PD patients was found^[54]. Another study found a positive correlation between olfactory deficit and OB volume both in patients with PD and controls. No correlation of olfactory performance and olfactory sulcus depth was found^[55]. Advanced MRI techniques, such as fMRI and DTI, may be a useful and objective diagnostic instrument to evaluate olfactory function and to support the discrimination of PD from healthy subjects, before onset

of motor symptoms (Table 2).

In particular, in effort to identify brain structures responsible for smell loss in PD, fMRI can be used to investigate brain activity related to olfactory processing in PD.

Table 2 Evaluation of olfactory function in Parkinson's disease (PD) by functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI)

MR technique	Disease	Result	Reference
fMRI	PD	Reduction of activity in amygdaloid complex and hippocampal	56-57
	Non-detectable OERPs PD patients	Reduction of activity in the anterior cingulate gyrus and the portions of the left striatum	
	Detectable OERPs PD patients	High activation of amygdala, parahippocampal cortex, inferior frontal gyrus, insula, cingulate gyrus, striatum, and inferior temporal gyrus	56
DTI	De novo PD	Low FA value in WM adjacent to gyrus rectus and surrounding primary olfactory cortex	58

OERPs: Olfactory event-related potentials.

For the initiation of fMRI and DTI measurements, a special system generator of olfactory stimuli is necessary. Based on the principles of air-dilution olfactometry, Kobal and Platting introduced a chemosensory stimulation with stimuli having a rectangular shape with rapid onset, precisely controlled in terms of timing, duration and intensity and not simultaneously activating other sensory systems^[59]. This can be achieved by the olfactometer which is a complex instrument for creation of well defined, reproducible smell or pain stimuli in the nose without tactile or thermal stimulation.

fMRI in PD patients indicated altered neuronal activity in the amygdaloid complex and hippocampal formation during olfactory stimulation^[56-57]. In addition, neuronal activity in components of cortico-striatal loops appeared to be up-regulated indicating compensatory processes involving the dopaminergic system^[57].

In a study combining fMRI and olfactory event-related potentials (OERPs) analysis in patients with PD, non-detectable OERPs patients exhibited reduced activity in the anterior cingulate gyrus and portions of the left striatum, while detectable OERPs patients exhibited higher activation, especially in the amygdala, parahippocampal cortex, inferior frontal gyrus (BA 47), insula, cingulate gyrus, striatum, and inferior temporal gyrus. The relationship between the expression of olfactory ERPs and cortical activation patterns seen during olfactory stimulation in fMRI in PD patients supports the idea that OERPs and fMRI brain activation study are sensitive markers of neurodegeneration in

olfactory regions, independent of the typically observed nigro-striatal degeneration in PD^[56].

A recent study has investigated by fMRI processing system of odors, demonstrating a difference between PD patients and controls, which depended on the type of stimulation (pleasant or unpleasant odor)^[60]. Pleasant and unpleasant stimulation led to lower activation in the amygdalo-hippocampal complex in patients compared to control. Pleasant stimuli enhanced activity in the striatum and left inferior frontal gyrus of patients, whereas unpleasant stimuli did not increase left inferior frontal gyrus. This study has shown a possible difference between PD patients and healthy control in emotional and conscious olfactory processing.

Evidence is emerging that diffusion imaging parameters might be altered in olfactory tract and SN in the early stages of clinical PD, possibly reflecting pathological changes. However, no study has examined olfaction and diffusion imaging in olfactory tract and SN in the same group of patients. One study compared newly diagnosed PD patients with a matched control group using both olfactory testing and DTI of the SN and anterior olfactory structures. Fourteen patients with stage 1-2 Hoehn & Yahr PD were matched to a control group by age and sex. All subjects completed the olfactory testing, as well as a series of MRI scans designed to examine diffusion characteristics of the olfactory tract and the SN. Olfactory testing revealed significant impairment in the patient group. DTI revealed significant group differences in both the SN and anterior olfactory region, with fractional anisotropy of the olfactory region clearly distinguishing the Parkinson's subjects from controls. This study has suggested that there may be value in combining behavioral (olfaction) and MRI testing to identify early PD^[61].

Another study of Ibarretxe-Bilbao *et al*^[58], has demonstrated that microstructural WM reductions are present in the central olfactory system of early stage PD patients and that these reductions are associated with reduced ability to smell. Indeed patients with severe microsmia and anosmia had lower FA values than PD patients with mild/moderate or no olfactory dysfunction and healthy controls in the WM adjacent to gyrus rectus. In addition, patients with anosmia had reduced FA in the WM surrounding primary olfactory areas in comparison with healthy controls. FA values in the WM adjacent to primary olfactory cortex and right gyrus rectus correlated with ability to smell in the PD group.

In conclusion, the detection of early olfactory dysfunction, which often predates clinical diagnosis and is common in PD, and the identification of brain structures responsible for smell loss may be useful biomarkers for prodromal PD.

CONCLUSION

The defining features of PD are characterized by their insidious onset and inexorable but variable progression. Reliable and well validated early markers for PD to

identify individuals “at risk” before motor and non motor symptoms, accurately diagnose individuals at the threshold of clinical PD, and monitor PD progression throughout its course would dramatically improve patient care and accelerate research into both PD cause and therapeutics. During the past two decades, much progress has been made in identifying and assessing PD markers, but as yet, no fully validated marker for PD is available. Nonetheless, there is increasing evidence advanced *in vivo* brain imaging and objective assessment of olfactory function will provide critical clues to assist in the early diagnosis and medical management of PD patients.

These methods are broadly defined as characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.

The lack of success of recent disease-modifying therapeutic trials coupled with the huge expense of other methods, such the nuclear medicine, has highlighted the need for such an ambitious approach to identify and validate early markers of PD progression for future clinical studies of disease-modifying drugs.

Author contributions: All authors contributed equally to the literature searches and the writing and revision of the manuscript.

Conflict of interest: None declared.

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(Edited by Bohnen N, Michetti F/Zhao LJ/Song LP)