Neuromelanin MR imaging in Parkinson's disease

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A B S T R A C T

The development and application of neuromelanin sensitive MR imaging has allowed the detection of significant changes in the substantia nigra (SN) of Parkinson's disease (PD) patients, with high sensitivity and specificity in differentiating PD patients from non-PD aged controls, even in early disease stages, namely at the time of clinical diagnosis. These MR neuromelanin changes in the SN of PD patients reproduced *in vivo* long known characteristic pathological changes of PD. Several image evaluation methods have been used, corroborating the reproducibility of the data and enabling wider applications of this imaging technique in the clinical practice.

In this review we analyze the background and the technical aspects of neuromelanin sensitive MR imaging, focusing on the applications of these specific sequences for the study of PD, emerging as a possible disease imaging biomarker and a promising tool for individual patient evaluation.

Keywords: Parkinson's Disease, neuromelanin, Substantia nigra, locus coeruleus, MRI.

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INTRODUCTION

Extensive developments in Magnetic Resonance (MR) imaging have profoundly changed the study of Parkinson's disease (PD), evolving from the role of merely excluding secondary parkinsonism to the possible emergence as a disease biomarker. MR advanced sequences in high field magnets opened the possibility to visualize the *substantia nigra* (SN) *in vivo* and to investigate specific PD pathological changes, enabling the development of high accuracy tools for disease diagnosis in early stages and for the comprehension of disease pathophysiology.

The depletion of dopaminergic neurons in the *substantia nigra* (SN) *pars compacta* (SNpc) and noradrenergic neurons in the *locus coeruleus* (LC), characteristic of PD (1), occurs early in the course of the disease and even in preclinical stages (2). The characteristic depletion of SN dopaminergic and LC noradrenergic neurons in PD (1-4) leads to a denervation of the striatum and additionally to extensive extra-nigral pathology (2). In PD the SN changes are not uniform being characterized by a more pronounced reduction of dopaminergic cells in the ventrolateral segment (5) with a corresponding loss of dopaminergic innervation in the posterior putamen (6).

Depigmentation of the SN and LC is a conspicuous pathological feature of PD, related to the loss of a melanin pigment known as **neuromelanin** (2). In spite of the long known characteristic depigmentation of the SN in PD being related to the loss of this pigment (2), the investigation of the role of neuromelanin in the pathophysiology of PD is a new field of research.

NEUROMELANIN AND PARKINSON'S DISEASE

Projection neurons from the SNc and LC are characterized by the presence of a dark colored pigment, related to skin melanin, called **neuromelanin** (7). Neuromelanin is a substance originating from dopamine and noradrenaline metabolism that is thought to have a protective role of neurons from oxidative stress mediated by free metals or free radicals (7,8). Following neuronal death neuromelanin can be released from neuropil (a complex network of axonal arborizations, dendrites and glia that forms most of the grey matter) or engulfed by macrophages (9).

The role of neuromelanin in the etiology and pathophysiology of PD was highlighted by several epidemiology studies that described an increased incidence of melanoma in PD patients and *vice versa* (10-14). The largest prospective melanoma study in PD, with a total of 2106 patients, reported a seven times increase of the relative risk of melanomas in this population (11). Another study associated the diagnosis of melanoma to a 50% increased risk of subsequent PD development (15) and data from a study that included 160 000 individuals

without neurological disease has shown that individuals with a familiar history of melanoma have more than a double risk of developing PD (13).

Epidemiology data additionally reports an increased incidence of PD (16-18) and of melanomas (19,20) in Caucasian populations compared to black individuals, indicating an increased vulnerability for the development of both skin tumors and PD associated with skin melanin concentration.

The notion that melanosis potentially protects against melanomas and PD is additionally supported by the negative association between tobacco smoke and the incidence of PD, consistently found in numerous studies over the last 50 years (21-23). Smoker's melanosis, a well documented phenomenon, is probably the result of increased melanin synthesis related to the stimulation of melanocytes by nicotine or the bonding of melanin to noxious tobacco substances (24).

Although the association between PD and melanomas is well documented the mechanisms of this association are not yet entirely known. The most accepted hypothesis implicates alpha-synuclein (α-Syn) which is known to play a crucial role in melanoma pathogenesis and PD.

The relationship between melanin and α-Syn, which is expressed in normal skin (25) and in melanomas (26), has opened new perspectives in the pathophysiological research of PD. The melanin pigments are derived from the aminoacid tyrosine and the enzymes tyrosinase (TYR) and tyrosine hydroxylase (TH) are involved in the biosynthesis of melanin and dopamine initiated by the conversion of tyrosine to DOPA (27,28). The finding that α-Syn can interact with TYR (29) and inhibit TH (27,30) raises the possibility that α-Syn can play a role in the regulation of the biosynthesis of both melanin and DA.

Therefore, in skin melanocytes of PD patients the increase in α-Syn inhibits TH decreasing melanin synthesis (26), with the consequent increase in melanoma risk. In dopaminergic neurons the interaction of TYR with increased levels of α-Syn changes the functions of this molecule inducing a toxic process with cytosolic DA and dopaquinone increased levels leading to mitochondrial damage and neuronal death (31). The association of α-Syn neurotoxicity with neuromelanin concentration changes (32,33) as a possible link between PD and melanoma has lead to a particular research interest in neuromelanin.

NEUROMELANIN-SENSITIVE MR IMAGING

In 2006 a Japanese group first described the application of a specific MR sequence that allowed the visualization of neuromelanin to the study of PD patients (34). The authors described a spontaneous high signal on specific T1-weighted MR imaging (34,35), related to the paramagnetic properties of this pigment allowing for the first time the *in vivo* visualization of important pathologic characteristics of PD.

Neuromelanin (NM)-sensitive MR imaging is a T1-weighted fast spin-echo (FSE) sequence, and in all published studies the parameters have only slight variations from the initial description by Sasaki et al (34). The vast majority of the neuromelanin PD studies were performed on 3.0 Tesla MR scanners, with only one report on 1.5T (36). 2D (34, 37-42) and 3D sequences have been used (36,43,44), with the latter enabling higher signal homogeneity and the possibility to perform volumetric measurements.

Neuromelanin signal was very hard to detect on MR and the developed NM-sensitive sequences have succeeded in demonstrating its presence through the combination of several factors especially the high signal-to-noise ratio (using 3.0T magnetic fields), the prolonged T1 relaxation time of the brain and an indirect magnetization transfer effect (via inter-slice cross-talk) (34). 2D FSE uses refocusing pulses which induce a magnetization transfer (MT) effect in off-resonance slices and this is important in NM-sensitive sequences to obtain contrast with the surrounding tissue, since the MT effect is reduced in neuromelanin (36).

Slice orientation is of critical importance to identify the LC and the SN signal and so the acquisition must be oriented perpendicular to the fourth ventricle floor, covering from the inferior border of the pons to the posterior commissure region (34) (Fig 1).

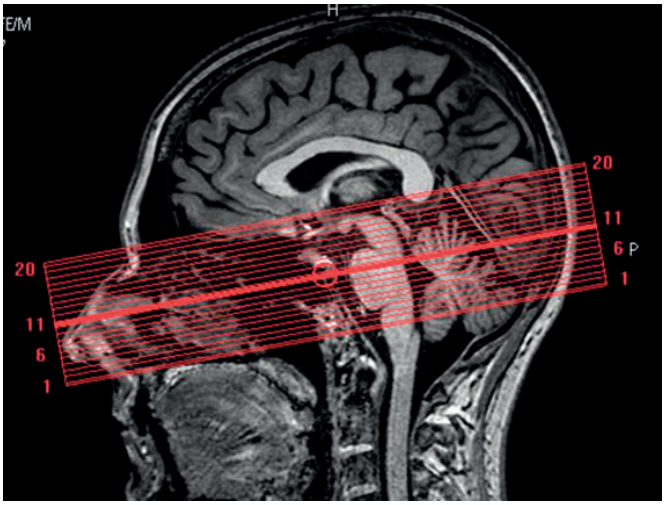

Figure 1.

NM-sensitive MR imaging slice positioning. Sections must be carefully set in the oblique axial plane perpendicular to the IV ventricle floor with coverage from the posterior commissure to the inferior border of the pons.

The sequence parameters have not varied significantly between the studies but several post processing methods have been used to analyze the obtained images. Manual tracing of regions-of-interest (ROIs) in the SN or LC high-intensity area (37,38,45,46), semi-automathed analysis (definition of high intensity areas in the SN with region-growing methods) (40,42,43), width measurements and simple visual inspection of the SN area (41) have been reported.

Authors have looked at the extent of the SN high-signal areas measuring areas/volumes (40,46) or analyzing signal intensity contrast ratios (37-39,45).

Manual tracing is time consuming but automated approaches need additional software, limiting availability.

The intra-rater reproducibility of semi-automated methods is good (43) but some of the steps are still operator dependent and so can not be fully automated. For routine use of neuromelanin imaging in clinical practice easy, fast and reproducible approaches must be implemented.

DO THESE T1-HIGH SIGNAL AREAS CORRESPOND HISTOLOGICALLY TO NEUROMELANIN?

In a recent study Kitao et al have made a direct correlation between pathology findings and neuromelanin MR imaging in Parkinsons's disease showing that the SN hyperintense areas in this sequence reflect neurons containing neuromelanin (47).

So this new imaging technique opened the possibility to study neuromelanin *in vivo* and has been applied in two main areas of research: the diagnosis of Parkinson's disease (possible emerging as a disease imaging biomarker) and in the differential diagnosis with other conditions, especially Essential tremor (ET) and atypical parkinsonian syndromes (APS).

1. NEUROMELANIN MR IMAGING FOR THE DIAGNOSIS OF PARKINSON'S DISEASE

PD becomes clinically manifest when the SN pathological changes have already reached an advanced stage (48) being estimated that a very significant degeneration of dopaminergic SN neurons has occurred before the beginning of motor symptoms (49). So by the time the patient has clinical complaints, most of the disease process has already occurred emphasizing the need for early and even pre-manifest disease markers, which can become very relevant for the implementation of an adequate therapy, for prognostic definition and even for the appropriate selection of patients in clinical trials (50).

Several studies have consistently demonstrated a reduction of neuromelanin high signal in the SN and the LC in PD (34, 38-43,45,46,51) (Fig 2). This is already apparent in early disease stages, and even at the time of clinical diagnosis (39-41,45). Interestingly most reports found a greater reduction in the lateral part of the SN with relative preservation in the medial segment (38,40,41,45), reproducing the known pathological characteristic of neuronal loss in PD (5). NM imaging was also used to study rapid eye movement sleep behavior in PD and the authors reported reduced signal intensity in the locus coeruleus /subcoeruleus area in patients with rapid eye movement compared to patients without those movements, concluding NM could be an early marker of non-dopaminergic Parkinson's disease pathology (44).

These NM measurements have a reported high sensitivity and specificity for PD patient differentiation from healthy controls (39,40,46,52), with high diagnostic accuracy even in early disease stages (40,43,45), only slightly lower than those obtained in [123]- FP-CIT SPECT studies in identifying PD patients (53,54).

WHAT HAPPENS TO NEUROMELANIN WITH DISEASE PROGRESSION?

There are, until now, still limited results of neuromelanin MR imaging to evaluate disease progression. Some reports have shown a stability of neuromelanin in the initial disease stages (40,42,45) in agreement with transcranial sonography studies in PD that have also demonstrated no significant changes of the ultrasound signal in the course of the disease (55). However, studies looking at the difference between early/advanced PD stages found a more pronounced reduction of neuromelanin in advanced disease stages (37,38,43,46). Miyoshi and colleagues found a gradual and stage-dependent reduction of neuromelanin in the medial SN of PD patients and a more intense reduction of neuromelanin LC signal in late-stage PD (38). This data shows that NM SN changes in PD begin ventrolaterally and extend medially (38,51) and so NM imaging may be a useful tool to evaluate disease progression in PD.

Correlations between NM and age, disease duration and UPDRS scores are inconsistent between the studies which may be related to the reduced sample sizes of most studies (42,46). The lack of correlation of neuromelanin MR measurements in the SN contralateral versus ipsilateral to the clinically most affected side (42) may also be related to

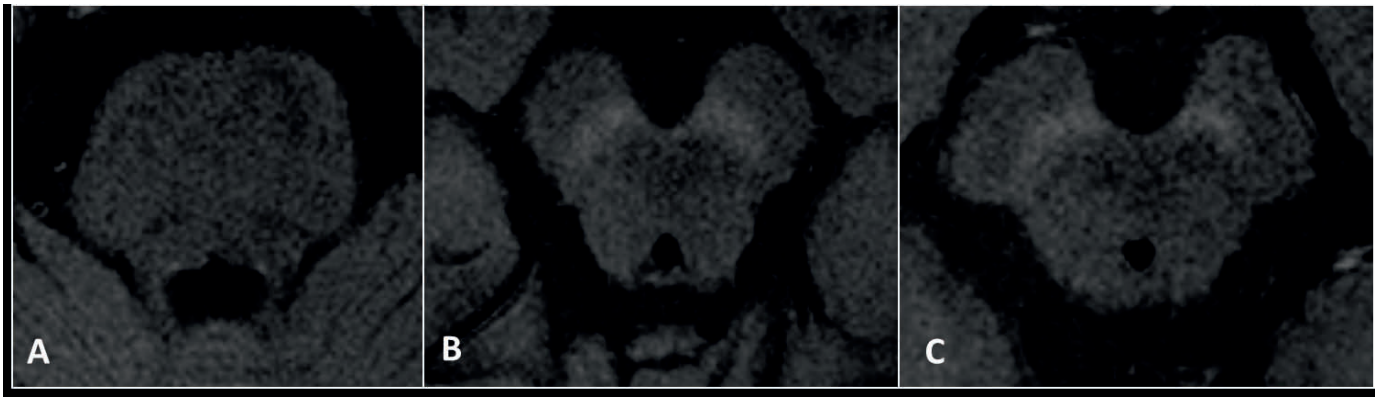

Figure 2.

Locus coeruleus (A) and substantia nigra (B) NM-sensitive MR imaging in a healthy subject and SN NM in a PD patient (C) (signal reduction in the lateral left SN).

the small sample sizes but can also reflect neuropathology data regarding asymmetries of the nigrostriatal system in patients with PD (56).

A recent neuromelanin MR study has looked at PD genetic forms (LRRK2 and PARKIN mutations) and found significant reduction of volumes in SNpc and LC in idiopathic PD (iPD) and LRRK2 and PARKIN mutation PD patients when compared to controls but no difference between iPD and mutation carriers (42). However the numbers are very small to allow a significant conclusion.

2. NEUROMELANIN MR IMAGING FOR THE DIFFERENTIAL DIAGNOSIS OF PARKINSON'S DISEASE

The diagnosis of PD is based on well established clinical criteria (57), however the differentiation with other causes of tremor and parkinsonism can be challenging, especially in early disease stages where a significant clinical overlap can occur (58). In atypical/uncertain cases the exclusive use of clinical criteria for PD diagnosis can have a high rate of misdiagnosis, even if performed by movement disorders specialized neurologists (59,60).

Neuromelanin sensitive MR imaging has been used in the differentiation of PD with **Essential tremor** (ET), which is very important in defining prognosis and treatment decisions and in identifying patients for research. A recent study showed that neuromelanin in the SN of ET patients was not significantly reduced compared with healthy controls, as opposed to tremor-dominant PD patients (61). The obtained high sensitivity and specificity for differentiation of ET from PD patients were only slightly lower than those obtained in [123]- FP-CIT SPECT studies in discriminating ET from PD even at the time of clinical diagnosis (53,54,62).

There are only a few studies using NM MR imaging to study **atypical parkinsonian syndromes**. An initial study found a marked reduction of neuromelanin signal in the SN and LC of early stage PD, multiple system atrophy (MSA) of parkinsonian type (MSA-P) and progressive supra-nuclear palsy (PSP) (63), but a more recent study has described a less severe reduction of neuromelanin signal in the LC of MSA-P and no apparent change in PSP-P compared to healthy individuals (39). More data is needed to investigate atypical parkinsonian syndromes but NM sensitive MR imaging may help in the discrimination of patients in early disease stages.

NEUROMELANIN AND IRON

It is known that iron content in the SN is increased in PD and seems to increase even more with disease progression (64-66). A significant amount of LC and SN iron is normally sequestered in NM granules (67) but in PD although individual granules have higher iron levels (68) loss of nigral neurons leads to a significant decrease of neuromelanin with iron being stored in Lewy bodies (69) and increasing ferritin load (70). Neuromelanin exhibits paramagnetic properties when bound to iron that result in the acceleration of T1 relaxation (71,72).

HOW DOES THIS INCREASED IRON LOAD INFLUENCE NM-RELATED CONTRAST?

A recent study combined NM sensitive MR imaging with iron quantification in the SN of early stage PD patients and found no significant correlation between the two parameters (73). This lack of correlation can be related to the significant reduction of iron bound to neuromelanin in PD with increased storage as ferritin, hemosiderin and in Lewy bodies but can also reflect distinct pathophysiological processes (73). This data is also in agreement with the neuropathology data from Kitao et al that also found that the NM signal intensity in the SNpc does not seem to be influenced by iron deposition (47).

FUTURE PERSPECTIVES

NM-sensitive MR imaging has some limitations that will need to be addressed and optimized, especially the long acquisition time, the relatively low spatial resolution and in-plane signal inhomogeneity, partial volume effects and insufficient coverage, which have a significant impact in all performed measurements, especially on the study of the LC due to its reduced size. The sequence is primarily used in 3.0Tesla MR scanners with an impact on availability and post processing methods need uniformity and additional reproducibility studies.

The correlation with age, disease duration, clinical severity, disease clinical asymmetry and clinical phenotypes all need future studies with greater samples. The investigation of genetic forms, disease late stages and therapeutical effects are additional investigation challenges.

CONCLUSIONS

Recently developed MR neuromelanin-sensitive imaging has enabled the *in vivo* evaluation of the SN and the detection of specific changes in Parkinson's disease, reproducing known pathological changes of the disease. These changes have a high sensitivity and specificity for disease diagnosis early in the disease course and for the differential diagnosis, namely with Essential tremor. Neuromelanin MR imaging may become a useful diagnostic tool with high sensitivity and specificity for the diagnosis of PD, even in early disease stages and emerge as a disease biomarker, improving diagnostic accuracy and aiding in the understanding of heterogeneity of clinical characteristics of PD. Definition of the role of NM-sensitive MR imaging in the diagnosis and management of PD will require additional studies.

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