Title: MOTOR ASSOCIATIONS OF IRON ACCUMULATION IN DEEP GREY MATTER NUCLEI IN PARKINSON’S DISEASE: A CROSS-SECTIONAL STUDY OF IRON-RELATED MRI SUSCEPTIBILITY

Running Title: Motor associations of iron accumulation in PD

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DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

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ABSTRACT

Objectives

To determine whether iron deposition in deep brain nuclei as assessed using high-pass filtered phase imaging is associated with motor deficits in Parkinson’s Disease (PD).

Methods

Seventy patients with mild-moderate PD and 20 age-gender-matched healthy volunteers (HV) underwent susceptibility-weighted imaging (SWI) on a 3T MRI scanner. Phase-shift (radians) changes in the deep brain nuclei were derived from high-pass filtered phase images and compared between groups. Correlations with motor severity *(UPDRS-III) were performed between HV and three PD sub-groups divided according to UPDRS-III scores using analysis of covariance, adjusting for age and regional area.

Results

PD patients had significantly (p<0.001) higher radians than HV bilaterally in the Putamen, Globus Pallidus and Substantia Nigra (SN). The SN contralateral to the most affected side showed higher radians (p<0.001) compared to the less affected side. SN radians positively correlated with UPDRS-III and bradykinesia-rigidity subscores, but not with tremor subscores. ANCOVA followed by post-hoc Bonferroni-adjusted pairwise comparisons revealed that SN radians were significantly greater in the PD subgroup with the highest UPDRS-III scores.

Conclusion

Increased nigral iron accumulation in PD appears to be associated with disease severity and correlates with clinical features of nigrostriatal dopaminergic neurodegeneration. This semi-quantitative in vivo iron assessment could prove useful for objective monitoring of PD progression, especially in trials of iron chelation therapies.
INTRODUCTION

Iron is thought to play an important role in PD neurodegeneration. Free ferrous iron (Fe^{2+}) acts as a catalyst in a reaction with hydrogen peroxide (Fenton reaction), producing highly toxic reactive oxygen species (ROS) that leads to oxidative stress-related damage to cellular components including proteins, lipids and DNA (1). Indeed, post-mortem and in vitro studies have demonstrated links between iron accumulation and the cardinal pathological features of Parkinson’s disease, the loss of dopamine neurons in the substantia nigra pars compacta (SNc) (2) and the presence of α-synuclein-rich Lewy bodies (3).

*In vivo* iron quantification using magnetic resonance imaging (MRI) relaxometry (T2*, R2*) or Susceptibility-Weighted Imaging (SWI) has consistently shown that there are increased SN iron levels in patients with PD relative to healthy volunteers (4-14), with more heterogeneous results seen in striatal regions such as caudate nucleus, putamen and globus pallidus (4-12). Furthermore several studies have reported a relationship between increased nigral iron accumulation, disease duration and motor severity (8-10, 14), although to our knowledge there are currently no cross-sectional studies that evaluate whether significant changes in iron accumulation exist between different PD stages in the midbrain or striatal nuclei. In addition, it remains unclear as to the relationship between iron accumulation and different motor features, with the two studies looking at tremor and akinetic/rigid aspects of disease yielding conflicting results (12, 13).

Consequently the goal of this study was to 1) evaluate differences in iron accumulation in deep brain nuclei between PD groups of varying motor severity and 2) to investigate the association between motor features and iron load. We used phase images obtained as part of an SWI protocol because they contain information on the tissue magnetic susceptibility distribution, a property shown to be influenced by ferritin and hemosiderin. Conventional relaxometry techniques are also affected by background field inhomogeneities such as tissue water content and air-tissue interfaces, thus phase images represent a more direct means to estimate iron (15).
METHODS

Subjects

Seventy non-demented mild-moderate stage PD patients and twenty age-gender-matched healthy volunteers (HV) were included in this study (see Table S1 for recruitment information). Diagnosis of PD was made by movement disorder specialists according to the PDUK Brain Bank Criteria (16),.

Data were collected as part of the TransEuro and PaMIR studies, funded by FP7 and Parkinson’s UK respectively and carried out in accordance with the Declaration of Helsinki, after approval from the National Research Ethics Service Committee. All participants gave written informed consent before participation.

Motor assessment

Motor features were measured using the Unified Parkinson’s Disease Rating Scale, part III (UPDRS-III). Patients were instructed to withdraw from medication 24 hours prior to assessment. Off-medication UPDRS-III scores were then subdivided into tremor (sum of items 15-18) and bradykinesia-rigidity subscores (sum of items 2-9 and 14). Levodopa-equivalent daily dose (LEDD) was calculated for all patients.

Susceptibility-Weighted Imaging

Susceptibility-weighted images were acquired on a 3T Siemens Magnetom Trio system with a 32-channel phased-array head coil running a T2-weighted 3D gradient-echo sequence (SWI: TR=28ms, TE=20ms, Flip Angle=15°, bandwidth=120 Hz/Px, matrix size=294*320, FoV=230*230mm, GRAPPA acceleration factor=2). 72 slices of 1.9mm thickness and slice gap of 20% were obtained in an interleaved order parallel to the anterior-posterior commissural line in the left-hand reference system. A small number of
participants (4PD, 7HV) underwent a modified SWI protocol in which 88 slices of 0.9mm thickness with a 20% slice gap were acquired. No significant differences in radians were found between protocols. Phase images were reconstructed automatically on the Siemens workstation (Syngo MR B17 software, SWI version 1), which included application of a 64*64 high-pass filter to remove low spatial frequency effects.

**Regions of Interest Analysis**

Manual ROI delineation was performed by a trained investigator on both PD and HV high-pass filtered phase images using SPIN (Signal Processing in Nuclear Magnetic Resonance; MRI institute, Detroit, Michigan). ROIs were hand drawn on a single axial slice and included the head of the caudate nucleus, putamen and globus pallidus (Figure 1). The SN was delineated on the 3rd axial slice ventral to the most dorsal aspect of the red nucleus. Subjects with microvascular lesions or physiological calcifications in the globus pallidus were not included in the analysis.

Mean phase-shift values, number of voxels, standard deviations and regional areas were extracted for each ROI unilaterally before averaging to obtain bilateral data. Phase-shift values were converted to radians according to the formula provided for the Siemens left-handed system: \[ \text{Radians} = (\phi - 2048)\pi / 2048 \] (17).

*Figure 1*

**Statistical Analysis**

All statistical analyses were performed using SPSS statistical software (version 22.0 SPSS Inc., Chicago, Illinois).

Group demographics and bilateral radians for each region were compared between PD and HV using independent t-tests. ROIs showing a significant difference were considered for analyses of covariance (ANCOVA) in which
PD patients, subdivided into 3 disease severity groups using UPDRS-III tertile calculations (Table 1) were compared to the HV group, adjusting for age and regional area and followed with post-hoc Bonferroni-adjusted pairwise comparisons.

To assess hemispheric iron load differences in the PD group, radians for the clinically most/least affected sides, as indicated by UPDRS-III laterality items, were compared for each region using independent t-tests.

Pearson’s correlation coefficient was used to assess relationships between each ROI and measures of disease severity (disease duration, UPDRS-III total, tremor and bradykinesia/rigidity). Correlations were considered significant only if p<0.05 following Benjamini-Hochberg FDR adjustment.
RESULTS

Participants

Clinical and demographic data are summarized in Table 1. There were no significant differences in age \( (p=0.347) \) between the PD and HV cohorts. Although the male:female ratio was higher in the PD group \( (p=0.039) \), no significant gender differences in iron accumulation were found.

As expected, PD subgroups exhibited significant differences in motor severity, disease duration, LEDD and H&Y scores (Table 1). There were no significant differences between subgroups for age or gender.

\(<\text{Table 1}>\)

Phase-shift analysis

Shapiro-Wilk's test revealed that putaminal and pallidal radians were not normally distributed \( (p<0.05) \). Square root transformations were therefore applied to the putamen and logarithmic transformation of the globus pallidus values. The PD group showed significantly higher radians in the putamen, globus pallidus and SN \( (p<0.001) \), as compared to HV (Fig 2). No significant difference was found in the caudate nucleus.

\(<\text{Figure 2}>\)

ANCOVA including HV and PD subgroups indicated a main effect of motor severity in the putamen \( (F=6.346, p<0.001) \), globus pallidus \( (F=7.998, p<0.001) \) and SN \( (F=65.008, p<0.001) \) (Table 2). Post-hoc Bonferroni-adjusted pairwise comparisons showed that radians in all three PD subgroups were significantly higher than HV in the globus pallidus \( (p=0.025, p<0.001\text{ and } p=0.001\text{ respectively}) \) and SN \( (p<0.001, p<0.001\text{ and } p<0.001\text{ respectively}) \) (Table 2). Putaminal radians were significantly higher in PD subgroups 1 and 2 \( (p=0.006, p<0.001) \) as compared to HV. Pairwise comparisons between the PD subgroups revealed significance only in the SN, that is, PD group 1 showed significantly lower radians as compared to PD groups 2 \( (p=0.049) \).
and 3 (p=0.001). Although PD group 3 showed the highest radians in the SN, no statistically significant difference was found when compared with PD group 2 (p=0.236) (Figure 3).

<Table 2>

<Figure 3>

Analysis of clinical laterality

The SN contralateral to the clinically most affected side showed higher radians (p<0.001) compared to the other, least affected, side. No other significant results were found.

Correlations with disease duration and motor severity

Pearson’s correlation analysis revealed significant positive correlations between SN radians and total UPDRS-III (r=0.420, p<0.001) and with bradykinesia-rigidity (r=0.407, p=0.001) but not with tremor subscores (r=0.219, p=0.071) (Figure 4). Correlations remained significant after Benjamini-Hochberg FDR correction for multiple comparisons.

Within the HV group, SN radians positively correlated with age (r=0.533, p=0.016).

<Figure 4>
DISCUSSION

This cross-sectional magnetic resonance imaging study evaluated regional iron deposition in deep brain nuclei, using high-pass filtered phase images to quantify average phase-shifts (radians) in a large cohort of PD patients as well as age-matched healthy volunteers.

Higher levels of ferric iron, as measured using radians, were found in the SN of PD patients relative to the HV group. Furthermore, when the PD groups were subdivided according to their UPDRS-III score, the SN showed increasing iron accumulation with motor severity. This result is in accordance with other recent longitudinal studies that have also shown changes in SN iron accumulation over time which correlate with changes in motor severity (19, 20).

Although the pathogenic significance of this is unclear, it is known that the SN is particularly vulnerable to abnormal increases in iron. Neuromelanin, which under normal conditions acts as a neuroprotective iron chelator, is released into the extracellular space as a result of nigral cell death. Extracellular neuromelanin then releases ferric iron, which in turn promotes oxidative stress, microglial activation and further neuronal death (18).

Increased iron deposition was also found in the putamen and globus pallidus, although no difference was found in the caudate nucleus. Results relating to striatal mineralisation have been highly variable, with several studies reporting increases (4, 7, 9, 11), reductions (5, 6) or normal levels (8, 10, 12) in PD. One possible explanation for this heterogeneity could be due to the presence of calcifications in the striatum, which may cause SWI and relaxometry techniques to underestimate iron levels. Recently however, Ulla et al (19) demonstrated in an early PD cohort, that whilst there was a significant increase in R2* signal in the caudal putamen between baseline and three year follow-up as compared to healthy volunteers, the rostral putamen remained at normal levels. Thus, the heterogeneity of results within the literature may be attributable to a rostro-caudal gradient of mineralisation, which in turn may be dependent on disease duration. In line with this, our data, though non-
significant, did show a trend for an initial increase of iron accumulation from low to moderate PD motor severity followed by a decrease from moderate to high motor severity in both the putamen and globus pallidus. Interestingly, this trend is in accordance with Ryvlin et al (5) who suggested that mineralization in these regions inversely correlates with disease duration when the duration of illness is above ten years. In contrast to the SN, it is unclear as to the mechanism behind striatal iron accumulation and further longitudinal studies will be needed before the reported discrepancies can be better explained and understood.

SN iron has previously been shown to strongly correlate with disease duration and motor severity (8-10, 14). Here, we show that this relationship is largely driven by the bradykinesia/rigidity features of the patient, rather than the tremor. The reason for this is that the bradykinesia and rigidity features of PD are thought to be a direct consequence of dopaminergic nigral neurodegeneration (22), which is associated with regional iron accumulation (2). In contrast, tremor does not only reflect dopaminergic nigro-striatal degeneration but may also be associated with serotoninergic and noradrenergic pathology (23). Indeed, we recently found that dopaminergic presynaptic terminal availability as well as storage capacity in the putamen and caudate nucleus were negatively associated with bradykinesia and rigidity scores, but not with tremor (Li and Lao-Kaim et al, unpublished observations).

In contrast, Bunzeck et al found a significant association between striatal iron accumulation and tremor symptomatology, suggesting that putaminal iron may be used as a predictor for tremor dominance in early PD (13). However, their measures were collected in the “on” medication state whilst our assessments were conducted in the “off” medication state in order to avoid the confounding effect of variable clinical benefit with respect to tremor. The use of high-pass filtered phase imaging may be of therapeutic interest. Iron chelators that cross the blood-brain barrier, such as Deferiprone, used mainly for peripheral haematological disorders, show a disease-modifying effect in animal models of PD (24) and now entering clinical trials. So far, two randomized clinical trials with this drug have been conducted in PD patients, showing small reductions in motor severity after 6-12 months of treatment.
without major medication-related side effects (25) (Martin-Bastida et al, unpublished observations). However, MR relaxometry data were not consistent between these studies in detecting different degrees of regional chelation. Given the higher specificity for detecting ferric compounds such as ferritin and hemosiderin (15), phase imaging may represent a more direct approach to monitoring the effects of iron chelators longitudinally.

There are some limitations with our study. First, we analyzed the slice with the highest iron-content in order to avoid background artifacts. Although no ROI size differences were found between PD and HV, this method may not reflect the iron content of the entirety of each nucleus. Second, we analyzed the SN without segmenting the pars compacta (SNc) from the pars reticulata (SNr). It is well documented that the SNc and SNr have differential levels of mineralization, however, segmentation could not be achieved accurately due to the inability of phase images to resolve the anatomical boundaries between these two subregions. This nigral subdivision would be possible with quantitative susceptibility mapping (QSM); nevertheless this technique could not be performed in the present study (26).

In summary, our results demonstrate that the degree of iron accumulation in PD is associated with disease severity in the SN but not in striatal sub-regions and that nigral iron is associated with the severity of bradykinesia and rigidity rather than tremor. Phase imaging techniques could be an important tool for monitoring clinical progression and establishing the efficacy and neuroprotective effects of those iron chelation therapies that are now entering clinical trials in PD.

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DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

None of the authors report any conflict of interest.

Figure Legends

Figure 1: Regions of interest delineated on axial slices of high-pass filtered phase images. A: Head of caudate nucleus (1), Putamen (2), Globus pallidus (3). B: Substantia nigra (4). C: Sagittal 3D MPRAGE T1-weighted image indicating level of ROI delineation.

Figure 2: Comparison of radians between PD (grey bars) and HV (white bars) CN= Caudate Nucleus; Put= Putamen; GP=Globus Pallidus; SN= Substantia Nigra. ***p<0.001, **p<0.01, *p<0.05.

Figure 3: Box and whisker plot displaying ANCOVA results for the SN.PD group 1 (UPDRS III=19.3 ± 3.11); PD group 2 (UPDRS III=29.27 ± 3.13); PD
group 3 = 44.60 ± 6.42). Ad-hoc Bonferroni-adjusted pairwise comparison significance levels indicated as follows: **p<0.001, *p<0.01, *p<0.05.

**Figure 4:** Pearson correlation of SN radians with UPDRS-III, bradykinesia-rigidity and tremor scores.
REFERENCES

Supporting information

**Table S1:** Study volunteer recruitment from TransEuro and PaMIR studies. PD = Parkinson’s disease volunteers, HV = healthy volunteers.