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

AUTHORS:

Antonio Martin-Bastida MD MSc[1], Nicholas P Lao-Kaim MSc[1]*, Clare Loane PhD[1, 2]*, Marios Politis MD MSc DIC PhD[1,3], Andreas A Roussakis MD[1], Natalie Valle-Guzman MSc[4], Zinovia Kefalopoulou MD PhD[5], Gesine Paul-Visse MD PhD[6], Håkan Widner MD PhD[7], Yue Xing PhD[8], Stefan T Schwarz MD MSc FRCR MRCP[8], Dorothee P Auer MD PhD FRCP[8], Thomas Foltynie BSc MBBS MRCP PhD[5], Roger A Barker BA MBBS MRCP PhD[9], Paola Piccini MD PhD FRCP[1]

Lao-Kaim NP and Loane C are both second co-authors.

[1] *Centre for Neurodegeneration and Neuroinflammation, Division of Brain Sciences, Imperial College London, London, United Kingdom, W12 0NN.*

[2] *Memory Research Group, Nuffield Department of Clinical Neurosciences, Medical Science Division. University of Oxford, United Kingdom*

[3] *Neurodegeneration Imaging Group, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom, SE5 9RT.*

[4] *John Van Geest Centre for Brain Repair, University of Cambridge, Cambridge, United Kingdom, CB2 0PY.*

[5] *Sobell Department of Motor Neuroscience, UCL Institute of Neurology, National Hospital for Neurology and Neurosurgery, London, United Kingdom, WC1N 3BG.*

[6] *Translational Neurology Group, Department of Clinical Sciences, Wallenberg Neuroscience Centre, Lund University, 221 84, Lund, Sweden.*

[7] *Division of Neurology, Department of Clinical Sciences, Lund University, Skåne University Hospital, Lund 22185, Sweden.*

[8] *Radiological Sciences, Division of Clinical Neuroscience, University of Nottingham, Queen's Medical Centre Nottingham, United Kingdom, NG7 2UH.*

[9] *Department of Clinical Neuroscience, University of Cambridge, UK; MRC Cognition and Brian Sciences Unit, University of Cambridge, United Kingdom, CB2 7EF.*

Correspondence to: Paola Piccini, Neurology Imaging Unit, Hammersmith Hospital, Imperial College London, London, UK. Tel: +44 (0) 208 383 3751. Email: paola.piccini@imperial.ac.uk.

Article word count: 3396

DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

None of the authors report any conflict of interest.

ABSTRACT

Objectives

To determine whether iron deposition in deep brain nuclei assessed using high-pass filtered phase imaging play a role in motor disease severity 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) in deep brain nuclei were derived from high-pass filtered phase images and compared between groups. Analysis of clinical laterality and correlations with motor severity *(UPDRS-III) were performed. Radians were compared 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 higher UPDRS-III scores as compared to both lowest UPDRS-III PD and HV groups (p<0.001).

Conclusion

Increased nigral iron accumulation in PD appears to be stratified according to disease motor severity and correlates with symptoms related to dopaminergic neurodegeneration. This semi-quantitative *in vivo* iron assessment could

prove useful for objective monitoring PD progression, especially in clinical trials concerning iron chelation therapies.

INTRODUCTION

Iron plays an important role in PD neurodegeneration. 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) (1) and the presence of α-synuclein-rich Lewy bodies (2). Free ferrous iron (Fe^{2+}) acts as a catalyst in a reaction with hydrogen peroxide (Fenton reaction), producing highly toxic reactive oxygen species (ROS) and ferric iron (Fe^{3+}) that leads to oxidative stress-related damage to cellular components including proteins, lipids and DNA (3). To relieve potential toxicity, excess ferrous iron within deep brain nuclei is accumulated and transported into the core of apoferritin molecules where it is oxidized and safely stored in high concentrations in ferric state (4). Since ferritin (iron-containing apoferritin) is highly paramagnetic and indeed one of the only non-heme iron molecules present in sufficient quantity to cause local field inhomogeneities (4, 5), magnetic resonance imaging (MRI) can be used to measure ferritin-bound ferric iron *in vivo*. Given that the biosynthetic rate of apoferritin is modulated by the availability of free iron (6, 7), changes in ferritin-related MR signal are reflective of alterations in iron load.

In vivo iron quantification using MRI relaxometry (T2*, R2*) or Susceptibility-Weighted Imaging (SWI) consistently demonstrates increased SN iron levels in patients with PD relative to healthy volunteers (8-18), while results pertaining to striatal regions such as caudate nucleus, putamen and globus pallidus appear highly heterogeneous (8-16). Several studies observe relationships between increased nigral iron accumulation, disease duration and motor severity (12-14, 18) though to our knowledge there are currently no cross-sectional studies that evaluate whether significant changes in iron accumulation exist between different stages of disease burden in mesencephalic or striatal nuclei. In addition, it remains unclear as to the effect of iron accumulation on differing motor symptoms, with the two studies to investigate tremor and akinetic/rigid predominance yielding conflicting results (16, 17).

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 symptomatology and iron load. We use 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 (breakdown product of ferritin) and which correlates with iron concentrations post-mortem (14, 19). 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 (4). This work demonstrates the value of iron quantification as an objective means by which patients can be monitored and stratified, particularly in the light of developing iron modifying therapies.

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 performed by movement disorder specialists according to the PDUK Brain Bank Criteria (20), excluding atypical parkinsonism, concomitant vascular load, history of cognitive impairment and neurodegenerative disorders other than PD.

Data were collected under 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 symptomatology was 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.

Susceptibility-Weighted Imaging

Susceptibility-weighted images were acquired on a 3T Siemens Magnetom Trio system with 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. 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 (AMB) on both PD and HV high-pass filtered phase images using SPIN (Signal Processing in Nuclear Magnetic Resonance; MRI institute, Detroit, Michigan). All study subjects were anonymised prior to delineation, thus blinding group and disease severity. ROIs were hand drawn on a single axial slice and included 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. For images acquired under the modified SWI protocol, the 5th axial slice ventral to the most dorsal aspect of the red nucleus was used (Figure S1). Independent samples t-tests showed no significant differences in SN radians between the two SWI protocols for either group (p>0.05) (Table S2). 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: *Radians=(ϕ-2048)π/2048* (21).

<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 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 PD and HV. 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.

<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 to globus pallidus. The PD group showed significantly higher radians in the putamen, globus pallidus and SN (p<0.001), as compared to HV (Figure 2). No significant difference was found in the caudate nucleus.

<Figure 2>

ANCOVA including HV and PD subgroups indicated main effects 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 and p=0.001 respectively) and SN (p<0.001, p<0.001 and p<0.001 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 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 bradykinesia-rigidity subscores (r=0.407, p=0.001) but not with tremor (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>

Validation of phase-shift data

To validate whether phase-shift data provide a quantitative measure of iron, Pearson's correlations were conducted between deep brain grey matter nuclei radians from the healthy volunteer group and distribution of non-heme iron at autopsy, as reported by Hallgren and Sourander (1958) (22). A positive

correlation was found after exclusion of CN due to spatial discordance with regards to regional iron measurements (r=0.874, p=0.01) (Figure S2).

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-shift (radians) in a large cohort of PD patients as well as age-matched healthy volunteers.

Higher levels of iron, as measured using radians, were found in the SN of PD patients relative to the HV group. Further, when PD groups were subdivided according to UPDRS-III score, the SN showed a stratified distribution in which iron accumulation increased with motor severity. The SN is particularly vulnerable to abnormal increases in iron. Neuromelanin (NM), 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 (23). Indeed, increased nigral iron has been largely demonstrated in imaging studies using both SWI and relaxometry techniques (8-18). Recent longitudinal studies have also shown that changes in SN iron accumulation over time correlate with changes in motor severity (24, 25). Our design provides further evidence that this effect is not limited to increases within individuals but that significant changes can be seen between patients at different stages of disease. We note that although there was an increase in iron from moderate to high PD groups, this was not significant, suggesting that the rate of accumulation may decrease as the disease progresses. These findings cannot be explained by age differences, which is well-known to correlate with nigral iron levels (26) nor between-patient nigral size, since both were added as covariates in our model.

Increased iron deposition was also found in the putamen and globus pallidus, though no difference was found in the caudate nucleus. Results relating to striatal mineralisation have been highly variable, with several studies reporting increases (8, 11, 13, 15), reductions (9, 10) or normal levels (12, 14, 16) 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 (24) 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 addition, our data, though non-significant, 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 (9) who suggested that mineralization in these regions inversely correlate with disease duration when the duration of illness is above ten years. In contrast to the SN, it is clear that the mechanism behind striatal iron accumulation is not yet understood. Further longitudinal studies with patients followed-up to the point of moderate to high motor severity are required to more accurately characterise the trajectory of striatal mineralisation in PD.

SN iron has previously been shown to strongly correlate with disease duration and motor severity (12-14, 18). Here, we add that this relationship may be driven by bradykinesia/rigidity symptomatology, since although a strong relationship was found between these measures no association with tremor was identified. Bradykinesia and rigidity symptoms result as a direct consequence of dopaminergic nigral neurodegeneration (27), which is associated with regional iron accumulation (1). In contrast, tremor does not only reflect dopaminergic nigro-striatal degeneration but may also be associated with serotoninergic and noradrenergic pathology (28). 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).* 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 (17). 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. Thus our data suggest that iron levels in the SN may be a feasible biomarker of bradykinesia and rigidity progression in PD.

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 (29). 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 (30) *(Martin-Bastida et al, unpublished observations)*. However, MR relaxometry data were not consistent between these studies, detecting different degrees of regional chelation. Conventional relaxometry techniques (T2* and R2*) estimate iron load *in vivo* using gradient-echo (GE) sequences that are sensitive to variations in transverse relaxation times/rates between different tissues and which are affected by both macroscopic and microscopic magnetic field inhomogeneities (31). However, images produced using these methods can be affected by changes in tissue water content and other background field inhomogeneities such as those resulting from air-tissue interfaces (4, 31). In contrast, SWI sequences, while also utilising T2* weighting involve the collection of both magnitude and phase images using a 3D velocity-compensated GE sequence. In particular, phase images contain information on the tissue magnetic susceptibility distribution. Since paramagnetic molecules such as ferritin and hemosiderin influence the local magnetic field and therefore the phase of the MR signal (31), phase offers greater specificity to quantifying brain iron load. Phase images are also more robust to the presence of noise (32) and the removal of low spatial frequency artefacts that could otherwise obscure intertissue phase differences (using a high-pass filter) (33) further improve its sensitivity to detecting abnormal iron levels. Thus, high-pass filtered phase images are a good alternative for 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, although it is well documented that the pars compacta (SNc) and pars reticulata (SNr) present differential levels of mineralization, we analyzed the SN without further segmentation. Post-mortem studies including those immunostaining for calbindin D_{28K} (34) have provided valuable information on the definition of nigral compartments based on dopamine cell-independent anatomical landmarks. However, translation to MRI segmentation is not straightforward due to technical considerations (acquisition volume, slice orientation and thickness) and imperfect correspondence between anatomically defined nigral distribution and that observed on T2-weighted images (35, 36). Nevertheless, attempts have been made to manually separate the nigra albeit with significant methodological variation (12, 24), highlighting disagreement on best localization procedures. More sophisticated techniques have recently been developed involving multimodal analysis of both SWI and NM-sensitive T1-weighted MRI (magnetization transfer) with which reduced NM pigmentation has been demonstrated in the SNc in PD with very high sensitivity and specificity (37, 38). However, the authors note a spatial overlap of 12.6±4.6% between the two SN volumes further reflecting the difficulty of accurately segmenting the SN (39). Given the inability of phase images to resolve intra-nigral boundaries, we opted to delineate the SN as a whole in order to maintain accuracy of our measurements, though we acknowledge that this was at the cost of specificity and conclusiveness with regards to the level of mineralization for each sub-region and their relationship to PD pathology. Higher MR field strength and the use of quantitative susceptibility mapping (40) which can further reduce phase image artefacts may provide greater spatial validity with respect to iron and improve the accuracy with which nigral compartments can be interrogated.

In summary, our results demonstrate that the degree of iron accumulation in PD is stratified according to 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 iron chelation therapies in PD patients of variable clinical phenotype.

ACKNOWLEDGEMENTS & FUNDING

DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

None of the authors report any conflict of interest.

REFERENCES

1.   Dexter DT, Wells FR, Agid F, et al. Increased nigral iron content in postmortem parkinsonian brain. *Lancet* 1987;**2**(8569):1219-20.

2.   Hashimoto M, Hsu LJ, Xia Y, et al. Oxidative stress induces amyloid-like aggregate formation of NACP/alpha-synuclein in vitro. *Neuroreport* 1999;**10**(4):717-21.

3.   Dexter DT, Carayon A, Javoy-Agid F, et al. Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* 1991;**114 (Pt 4)**:1953-75.

4.   Haacke EM, Cheng NY, House MJ, et al. Imaging iron stores in the brain using magnetic resonance imaging. *Magnetic Resonance Imaging* 2005;**23**(1):1-25.

5.   Schenck JF, Zimmerman EA. High-field magnetic resonance imaging of brain iron: birth of a biomarker? *NMR in Biomedicine* 2004;**17**(7):433-45.

6.   Casey JL, Hentze MW, Koeller DM, et al. Iron-responsive elements: regulatory RNA sequences that control mRNA levels and translation. *Science* 1988;**240**(4854):924-8.

7.   Munro HN, Linder MC. Ferritin: structure, biosynthesis, and role in iron metabolism. *Physiological Reviews* 1978;**58**(2):317-96.

8.   Antonini A, Leenders KL, Meier D, Oertel WH, Boesiger P, Anliker M. T2 relaxation time in patients with Parkinson's disease. *Neurology* 1993;**43**(4):697-700.

9.   Ryvlin P, Broussolle E, Piollet H, Viallet F, Khalfallah Y, Chazot G. Magnetic resonance imaging evidence of decreased putamenal iron content in idiopathic Parkinson's disease. *Archives of Neurology* 1995;**52**(6):583-8.

10.   Graham JM, Paley MN, Grunewald RA, Hoggard N, Griffiths PD. Brain iron deposition in Parkinson's disease imaged using the PRIME magnetic resonance sequence. *Brain* 2000;**123 Pt 12**:2423-31.

11.   Kosta P, Argyropoulou MI, Markoula S, Konitsiotis S. MRI evaluation of the basal ganglia size and iron content in patients with Parkinson's disease. *Journal of Neurology* 2006;**253**(1):26-32.

12.   Martin WR, Wieler M, Gee M. Midbrain iron content in early Parkinson disease: a potential biomarker of disease status. *Neurology* 2008;**70**(16 Pt 2):1411-7.

13.   Wallis LI, Paley MN, Graham JM, et al. MRI assessment of basal ganglia iron deposition in Parkinson's disease. *Journal of Magnetic Resonance Imaging* 2008;**28**(5):1061-7.

14.   Zhang J, Zhang Y, Wang J, et al. Characterizing iron deposition in Parkinson's disease using susceptibility-weighted imaging: an in vivo MR study. *Brain Research* 2010;**1330**:124-30.

15.   Wang Y, Butros SR, Shuai X, et al. Different iron-deposition patterns of multiple system atrophy with predominant parkinsonism and idiopathic Parkinson diseases demonstrated by phase-corrected susceptibility-weighted imaging. *AJNR: American Journal of Neuroradiology* 2012;**33**(2):266-73.

16.   Jin L, Wang J, Jin H, et al. Nigral iron deposition occurs across motor phenotypes of Parkinson's disease. *European Journal of Neurology* 2012;**19**(7):969-76.

17.   Bunzeck N, Singh-Curry V, Eckart C, et al. Motor phenotype and magnetic resonance measures of basal ganglia iron levels in Parkinson's disease. *Parkinsonism & Related Disorders* 2013;**19**(12):1136-42.

18.   Wu SF, Zhu ZF, Kong Y, et al. Assessment of cerebral iron content in patients with Parkinson's disease by the susceptibility-weighted MRI. *European Review for Medical and Pharmacological Sciences* 2014;**18**(18):2605-8.

19.   Hopp K, Popescu BF, McCrea RP, et al. Brain iron detected by SWI high pass filtered phase calibrated with synchrotron X-ray fluorescence. *Journal of Magnetic Resonance Imaging* 2010;**31**(6):1346-54.

20.   Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *Journal of Neurology, Neurosurgery and Psychiatry* 1992;**55**(3):181-4.

21.   Haacke EM, Miao Y, Liu M, et al. Correlation of putative iron content as represented by changes in R2* and phase with age in deep gray matter of healthy adults. *Journal of Magnetic Resonance Imaging* 2010;**32**(3):561-76.

22.   Hallgren B, Sourander P. The effect of age on the non-haemin iron in the human brain. *Journal of Neurochemistry* 1958;**3**(1):41-51.

23.   Zecca L, Tampellini D, Gerlach M, Riederer P, Fariello RG, Sulzer D. Substantia nigra neuromelanin: structure, synthesis, and molecular behaviour. *Molecular Pathology* 2001;**54**(6):414-8.

24.   Ulla M, Bonny JM, Ouchchane L, Rieu I, Claise B, Durif F. Is R2* a new MRI biomarker for the progression of Parkinson's disease? A longitudinal follow-up. *PloS One* 2013;**8**(3):e57904.

25.   Wieler M, Gee M, Martin WR. Longitudinal midbrain changes in early Parkinson's disease: iron content estimated from R2*/MRI. *Parkinsonism & Related Disorders* 2015;**21**(3):179-83.

26.   Pfefferbaum A, Adalsteinsson E, Rohlfing T, Sullivan EV. MRI estimates of brain iron concentration in normal aging: comparison of field-dependent (FDRI) and phase (SWI) methods. *Neuroimage* 2009;**47**(2):493-500.

27.   Vingerhoets FJ, Schulzer M, Calne DB, Snow BJ. Which clinical sign of Parkinson's disease best reflects the nigrostriatal lesion? *Annals of Neurology* 1997;**41**(1):58-64.

28.   Loane C, Wu K, Bain P, Brooks DJ, Piccini P, Politis M. Serotonergic loss in motor circuitries correlates with severity of action-postural tremor in PD. *Neurology* 2013;**80**(20):1850-5.

29.   Dexter DT, Statton SA, Whitmore C, et al. Clinically available iron chelators induce neuroprotection in the 6-OHDA model of Parkinson's disease after peripheral administration. *J Neural Transm (Vienna)* 2011;**118**(2):223-31.

30.   Devos D, Moreau C, Devedjian JC, et al. Targeting chelatable iron as a therapeutic modality in Parkinson's disease. *Antioxid Redox Signal* 2014;**21**(2):195-210.

31.   Haacke EM, Xu Y, Cheng YC, Reichenbach JR. Susceptibility weighted imaging (SWI). *Magnetic Resonance in Medicine* 2004;**52**(3):612-8.

32.   Haacke EM, Ayaz M, Khan A, et al. Establishing a baseline phase behavior in magnetic resonance imaging to determine normal vs. abnormal iron content in the brain. *Journal of Magnetic Resonance Imaging* 2007;**26**(2):256-64.

33.   Haacke EM, Mittal S, Wu Z, Neelavalli J, Cheng YC. Susceptibility-weighted imaging: technical aspects and clinical applications, part 1. *AJNR: American Journal of Neuroradiology* 2009;**30**(1):19-30.

34.   Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D(28K) immunohistochemistry. *Brain* 1999;**122 (Pt 8)**:1421-36.

35.   Sasaki M, Shibata E, Tohyama K, et al. Monoamine neurons in the human brain stem: anatomy, magnetic resonance imaging findings, and clinical implications. *Neuroreport* 2008;**19**(17):1649-54.

36.   Oikawa H, Sasaki M, Tamakawa Y, Ehara S, Tohyama K. The substantia nigra in Parkinson disease: proton density-weighted spin-echo and fast short inversion time inversion-recovery MR findings. *AJNR: American Journal of Neuroradiology* 2002;**23**(10):1747-56.

37.   Sasaki M, Shibata E, Tohyama K, et al. Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. *Neuroreport* 2006;**17**(11):1215-8.

38.   Schwarz ST, Rittman T, Gontu V, Morgan PS, Bajaj N, Auer DP. T1-weighted MRI shows stage-dependent substantia nigra signal loss in Parkinson's disease. *Movement Disorders* 2011;**26**(9):1633-8.

39.   Langley J, Huddleston DE, Chen X, Sedlacik J, Zachariah N, Hu X. A multicontrast approach for comprehensive imaging of substantia nigra. *Neuroimage* 2015;**112**:7-13.

40.   Barbosa JH, Santos AC, Tumas V, et al. Quantifying brain iron deposition in patients with Parkinson's disease using quantitative susceptibility mapping, R2 and R2. *Magnetic Resonance Imaging* 2015;**33**(5):559-65.

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 $\pm$ 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's correlations of SN radians with UPDRS-III, bradykinesia-rigidity and tremor scores.

SUPPLEMENTARY INFORMATION LEGENDS

Figure S1: Axial slices of two different subjects comparing SN delineation between the main (slice thickness =1.9, no. of slices =72) and modified (slice thickness =0.9, no. of slices =88) SWI protocols. For the main SWI protocol, delineation was conducted on the 3rd axial slice ventral to the most dorsal aspect of the red nucleus (left). For the modified SWI protocol, delineation was conducted on the 5th axial slice ventral to the most dorsal aspect of the red nucleus (right).

Figure S2: Pearson's correlations between phase-shift radians of deep brain grey matter nuclei from the healthy volunteer group (n=20) with the distribution of non-heme iron at autopsy (mg iron/100 gr fresh tissue), as reported by Hallgren and Sourander (1958). In addition to the putamen (Put), globus pallidus (GP), Substantia Nigra (SN) and head of the caudate nucleus (CN), radians were also derived from the thalamus (Tha), red nucleus (RN) and dentate nucleus (DN). After exclusion of CN as an outlier, we found a positive correlation between radians and non-heme iron (mg/100 g fresh weight) (r=0.874, p=0.01) (left). We hypothesised that the appearance of the CN as an outlier was related to a regional discordance; while Hallgren and Sourander (1958) reported non-heme values for the whole caudate, in this manuscript we originally reported values specifically for the caudate head only, which we observed had the highest iron values within the whole nucleus. We therefore also analysed the body of the caudate (CNb) and re-ran the correlation, substituting CN for CNb. Again we found a significant correlation between radians and non-heme iron (mg/100 g fresh weight) (r=0.624, p=0.034) (right).

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

Table S2: Independent samples t-tests comparing SN radians between the main SWI protocol 1 (slice thickness =1.9, no. of slices =72) and the modified SWI protocol 2 (slice thickness =0.9, no. of slices =88) for HV and PD groups. Values represent the mean±SD.