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Decreased serum ceruloplasmin levels characteristically aggravate nigral iron deposition in Parkinson's disease

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In vivo **and post-mortem studies have demonstrated that increased nigral iron content in patients with Parkinson's disease is a prominent pathophysiological feature. However, the mechanism and risk factors associated with nigral iron deposition in patients with Parkinson's disease have not been identified and represent a key challenge in understanding its pathogenesis and for its diagnosis. In this study, we assessed iron levels in patients with Parkinson's disease and in age- and gender-matched control subjects by measuring phase values using magnetic resonance based susceptibility-weighted phase imaging in a 3T magnetic resonance system. Phase values were measured from brain regions including bilateral substantia nigra, globus pallidus, putamen, caudate, thalamus, red nucleus and frontal white matter of 45 patients with Parkinson's disease with decreased or normal serum ceruloplasmin levels, together with age- and gender-matched control subjects. Correlative analyses between phase values, serum ceruloplasmin levels and disease severity showed that the nigral bilateral average phase values in patients with Parkinson's disease were significantly lower than in control subjects and correlated with disease severity according to the Hoehn and Yahr Scale. The Unified Parkinson's Disease Rating Scale motor scores from the clinically most affected side were significantly correlated with the phase values of the contralateral substantia nigra. Furthermore, nigral bilateral average phase values correlated highly with the level of serum ceruloplasmin. Specifically, in the subset of patients with Parkinson's disease exhibiting reduced levels of serum ceruloplasmin, we found lowered nigral bilateral average phase values, suggesting increased nigral iron content, while those patients with normal levels of serum ceruloplasmin exhibited no changes as compared with control subjects. These findings suggest that decreased levels of serum ceruloplasmin may specifically exacerbate nigral iron deposition in patients with Parkinson's disease. Combining susceptibility-weighted phase imaging with serum ceruloplasmin determination is likely to be useful for the diagnosis and assessment of a subset of patients with Parkinson's disease.**

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Abbreviations: SWI = susceptibility-weighted imaging; UPDRS = Unified Parkinson's Disease Rating Scale

Introduction

Parkinson's disease is a common movement disorder that manifests with varying combinations of rest tremors, bradykinesia, rigidity and postural instability. The dominant pathological hallmark is degeneration and loss of the dopaminergic neurons in the substantia nigra of the midbrain (Damier *et al.,* 1999; Braak *et al.,* 2003). Increased nigral iron content in patients with Parkinson's disease is also a prominent pathophysiological feature involved in selective dopaminergic neurodegeneration, which has been consistently demonstrated post-mortem and *in vivo* (Dexter *et al.,* 1987, 1991; Graham *et al.,* 2000; Zecca *et al.,* 2004; Berg and Hochstrasser, 2006; Rhodes and Ritz, 2008).

Iron plays important roles in many biological processes ranging from facilitating cellular aerobic metabolism to participating in signal transduction and synthesis of neurotransmitters and DNA (Loeffler *et al.,* 1995; Altamura and Muckenthaler, 2009). However, iron accumulation in the brain can be detrimental to neurons because it produces alterations in the ratio of ferric to ferrous iron, leading to the generation of toxic hydroxyl radicals (Bharucha *et al.,* 2008). Furthermore, iron deposition in the brain can also promote conformational changes in α-synuclein, resulting in its aggregation and contributing to the pathogenesis of Parkinson's disease (Graham *et al.,* 2000). Together, these studies suggest that iron deposition contributes to the mechanism of brain damage in patients with Parkinson's disease (Loeffler *et al.,* 1995; Jenner, 2003; Zecca *et al.,* 2008).

To date, the risk factors associated with nigral iron deposition in Parkinson's disease have not been identified and represent a key challenge to understand the pathogenesis and to diagnose it. Ceruloplasmin is a copper-containing protein with ferroxidase function (Vassiliev *et al.,* 2005). Under normal conditions, ceruloplasmin oxidizes ferrous iron into the ferric form, keeping the level of dangerous ferrous iron within the cell to a minimum (Osaki *et al.,* 1966; Lee *et al.,* 1968), thus playing an important role in iron metabolism. Mutation in the ceruloplasmin gene, resulting in the absence of circulating serum ceruloplasmin, leads to hereditary aceruloplasminaemia (Okamoto *et al.,* 1996). Interestingly, the clinical pathology of this disorder, which is characterized by neurodegeneration and retinal degeneration, is not related to copper deficiency/toxicity but due to substantial iron accumulation in the basal ganglia and retina (Harris *et al.,* 1995; Morita *et al.,* 1995). Supporting this, Patel *et al*. (2002) also demonstrated that increased iron accumulation and free radical injury occurred in the central nervous system of ceruloplasmin$^{-/-}$ mice.

Our previous study demonstrated that decreased serum ceruloplasmin levels, independent of the ATP7B mutation, are related to movement disorders, including Parkinson's disease (Lirong *et al.,* 2009). Torsdottir *et al*. (1999) have also previously reported that a group of patients with Parkinson's disease had on average significantly lower serum ceruloplasmin levels compared with that of age- and gender-matched controls. Consistently, Hochstrasser *et al*. (2004) demonstrated that patients with Parkinson's disease with ceruloplasmin mutations exhibited ultrasound hyperechogenicity, indicative of increased iron levels in the substantia nigra. These authors also demonstrated that ceruloplasmin mutations found in patients with Parkinson's disease affect iron metabolism in cultured cells (Hochstrasser *et al.,* 2005). Thus, disrupted ceruloplasmin metabolism probably represents a risk factor for Parkinson's disease by increasing brain iron levels.

Although previous work has examined iron levels in the brain of patients with Parkinson's disease, the association between iron deposition in specific brain regions and serum ceruloplasmin levels has not been determined. Recently, improved MRI techniques, such as susceptibility-weighted imaging (SWI), made it possible to assess brain iron deposition accurately. Brain iron stores alter both the magnitude and phase images of the magnetic resonance signal by creating subvoxel magnetic inhomogeneities. These changes can be quantitatively measured *in vivo* using magnetic resonance imaging (Haacke *et al.,* 2005). SWI is a 3D, high-resolution, fully flow-compensated gradient echo sequence that uses magnitude and phase data, both separately and together, to increase the information obtained from local tissue susceptibility variations. Its excellent phase shift at high magnetic fields has been well documented, in particular for imaging brain vessels and iron stores (Schuff, 2009). SWI is very sensitive to iron in the forms of haemosiderin, ferritin and deoxyhaemoglobin, making it possible to measure iron on the order of just 1 μg/g tissue *in vivo* (Haacke *et al.,* 2009). Several previous studies have demonstrated correlations between SWI phase shifts and brain iron concentration in various brain regions of healthy adults (Haacke *et al.,* 2007; Xu *et al.,* 2008; Pfefferbaum *et al.,* 2009). Thus, measuring the phase values of SWI at high magnetic fields represents a promising method for quantitatively assessing iron deposition in specific brain regions of patients with Parkinson's disease.

In this study, we utilized susceptibility-weighted phase imaging to investigate the phase values in various brain regions of patients with Parkinson's disease with decreased or normal serum ceruloplasmin levels and age- and gender-matched control subjects. The main objective of this study is to explore whether decreased serum ceruloplasmin levels are correlated with characteristic iron deposits in selective brain regions of patients with Parkinson's disease. This question is key to understanding the role of ceruloplasmin metabolism in modulating iron deposition in the brain and to determining whether decreased serum ceruloplasmin is a risk factor for Parkinson's disease.

Subjects and methods

Subjects

From April 2008 to March 2010, 242 patients with Parkinson's disease from a movement disorder clinic were identified and their serum

ceruloplasmin levels were screened. Patients with decreased serum ceruloplasmin (<0.20 g/l) accounted for 30.16% (73/242) of the total patients. Patients with Parkinson's disease were diagnosed by neurologists specializing in movement disorders (C.J.Z. and L.R.J.) according to the criteria of the United Kingdom Parkinson's Disease Society Brain Bank for idiopathic Parkinson's disease (Gelb *et al.*, 1999) and found to be at stages I-III on the Hoehn and Yahr Scale. The following patients were excluded from this study: (i) patients with signs of upper and/or lower motor neuron dysfunction; (ii) patients with orthostatic hypotension within three years of Parkinson's disease onset; (iii) patients with cognitive impairment as assessed by the Mini-Mental State Examination (MMSE); and (iv) patients with hepatic and/or renal dysfunction.

Forty-five patients with Parkinson's disease who fulfilled the diagnostic criteria described above voluntarily enrolled in this study. Among these patients were 25 with decreased serum ceruloplasmin (serum ceruloplasmin <0.20 g/l) and 20 with normal serum ceruloplasmin (serum ceruloplasmin ≥0.20 g/l). The purpose of the study was explained to all patients and control subjects (see below). All subjects were informed that their participation in this study was unpaid and that written, informed consent for this study was needed. This study was approved by the ethical committee of Zhongshan Hospital, Fudan University.

Of these 45 Parkinson's disease patients, 27 had received medication for Parkinson's disease, the remaining 18 had early-phase Parkinson's disease and had not been previously medicated. On the day of the magnetic resonance scanning, Part III of the Unified Parkinson's Disease Rating Scale (UPDRS) was used to determine the severity of motor symptoms during optimal medication benefit or 'ON phase' with dopaminergic medication, which consisted of a single drug or a combination of levodopa, dopamine agonists (piribedil or pramipexole), monoamine oxidase B inhibitors (selegiline), catechol-*O*-methyl transferase inhibitors (entacapone) and trihexyphenidyl.

Forty-five age- and gender-matched control subjects were voluntarily recruited. All control subjects had no history of neurologic/psychiatric disorders and no abnormal signal in structural magnetic resonance imaging. To exclude significant cognitive impairment, all control subjects were assessed with the MMSE and had a total score >28. Demographic and clinical data are summarized in Table 1. There was no significant difference in age and sex between patients with Parkinson's disease and control subjects.

Serum ceruloplasmin was measured in all patients and control subjects using immunonephelometry kits (N antiserum against human ceruloplasmin, Dade Behring, Marburg, Germany) according to manufacturer's instructions, 1–3 days before the magnetic resonance scanning. Patients with decreased serum ceruloplasmin levels were retested. Data was included in the final sample set only if serum ceruloplasmin levels varied by <10% between the two measurements.

Magnetic resonance imaging protocol

All magnetic resonance images were obtained using a 3.0T system (Signa HDx, GE Medical System, Milwaukee, USA) equipped with an eight-channel head coil. The head was immobilized in the head coil with foam padding. First, sagittal T_1-weighted images were acquired with a fast spin-echo sequence to locate the precise positions of the anterior and posterior commissures. Second, conventional axial T_1- and T_2-weighted images were acquired for screening of space-occupying lesions and cerebrovascular diseases in the basal ganglia and mesencephalon. Routine magnetic resonance scanning covered the whole brain.

Finally, a 3D fast gradient echo images sequence was used to obtain the susceptibility-weighted magnetic resonance images. The susceptibility-weighted magnetic resonance images were taken parallel to the anterior–posterior commissural line and covered the nuclei of the basal ganglia and mesencephalon. Susceptibility-weighted phase imaging used the following parameters: repetition time = 36 ms, echo time = 20 ms, flip angle = 20°. A total of 28 slices were collected with a slice thickness of 2 mm and a matrix size of 320 × 256 ($N_x \times N_y$). Field of view = 24 cm. Flow compensation was applied. Both phase and magnitude images were acquired, but only phase data were used in further analyses.

Image processing

The raw data were transferred to a separate workstation (ADW4.3, GE) where the phase map was processed by a self-coded programme to obtain the filtered phase image. The phase images were filtered with a high-pass filter in order to create a new phase map that was called the corrected phase image (Fig. 1). In this study, the high-pass filter was performed by using a filter with a central matrix size of 32 × 32 to remove background field inhomogeneities. The detailed methods of image processing used were from Haacke *et al.* (2004). The phase values of the regions of interest were measured on the corrected phase images, which ranged from $-\pi$ to $+\pi$.

Image analysis

The regions of interest of the nuclei were drawn according to the anatomical structures, while the regions of interest in frontal white matter were circular (80 mm^2, 364 pixels). The regions of interest included the bilateral substantia nigra, red nucleus, globus pallidus, putamen, head of caudate, thalamus and frontal white matter (Fig. 1). Data for each nucleus were obtained from all visible slices, except for frontal white matter, where two consecutive slices were used. A trained radiologist, blinded to subject data, manually traced the regions of interest. All the regions of interest were remeasured two weeks later by the same person on the same images. The final values were the means of the two measurements.

To demonstrate the reproducibility of SWI measurements between scans, 10 patients and 10 control subjects were scanned twice in the same magnetic resonance imaging system. The results were compared and found to be highly consistent between scans (Supplementary Tables 1 and 2).

Statistical analysis

The demographic data of the patients and control subjects were compared using independent two-tailed *t*-test. Regional phase values were compared for different brain regions of patients and control subjects for each region using independent two-tailed *t*-tests or Mann–Whitney U-tests, depending on the normality of the distribution; Bonferroni correction was used for comparison between multiple groups. Covariance (ANOVA) analysis was used to compare regional phase values and patient subtypes, adjusted for age and gender. Lateralized motor scores were derived from the sum of lateralized features in the motor subscale of the UPDRS. To investigate the correlation of phase value in each brain region with age, onset age, duration of Parkinson's disease, UPDRS score, lateralized UPDRS motor score, 'medicated or not' and serum ceruloplasmin levels, bivariate (Pearson's) correlation and partial correlation analysis were used. Statistical significance is determined as $P < 0.05$, except for the Bonferroni correction, where $P < 0.005$. All statistical analyses were

Table 1 Clinical data and the effects of serum ceruloplasmin on bilateral average phase values of different brain regions

Patient group					Control group		
	PD group with normal CP levels (≥0.20)	PD group with decreased CP levels (<0.20)	Total/average				
			Values	Variability[a]	Values	Variability[a]	*P*-value
Number (M/F)	20 (14/6)	25 (17/8)	45 (31/14)	–	45 (26/19)	–	0.508*
Age (years)	58.10 ± 8.82	54.89 ± 12.24	56.31 ± 10.86	–	55.36 ± 14.90	–	0.729*
Age at onset (years)	55.94 ± 8.80	50.65 ± 12.61	52.82 ± 11.11	–		–	0.119**
Serum CP (g/l)	0.221 ± 0.019	0.169 ± 0.021	0.192 ± 0.033	–	0.231 ± 0.036	–	< 0.001***
MMSE	29.12 ± 1.09	29.40 ± 1.35	29.24 ± 1.21	–	29.56 ± 0.79	–	0.278*
UPDRS score	13.60 ± 8.87	16.80 ± 11.06	15.11 ± 9.93	–	–	–	0.436**
UPDRS motor score	10.85 ± 5.84	12.92 ± 8.01	12.02 ± 7.11	–	–	–	0.629**
Bilateral average phase values (radians)							
SN	−0.166 ± 0.029	−0.202 ± 0.028	−0.186 ± 0.033	0.177	−0.159 ± 0.028	0.176	< 0.001*, <0.001***
RN	−0.130 ± 0.045	−0.141 ± 0.049	−0.136 ± 0.047	0.346	−0.125 ± 0.041	0.328	0.247*, 0.300***
PU	−0.067 ± 0.032	−0.075 ± 0.042	−0.071 ± 0.038	0.535	−0.071 ± 0.032	0.451	0.768*, 0.472***
GP	−0.101 ± 0.027	−0.115 ± 0.041	−0.109 ± 0.036	0.330	−0.103 ± 0.023	0.223	0.348*, 0.174***
CA (head)	−0.098 ± 0.030	−0.096 ± 0.037	−0.097 ± 0.033	0.340	−0.092 ± 0.017	0.185	0.396*, 0.668***
TH	−0.008 ± 0.007	−0.012 ± 0.011	−0.010 ± 0.009	0.900	−0.011 ± 0.012	1.091	0.271*, 0.548**
FWM	0.005 ± 0.011	0.006 ± 0.012	0.006 ± 0.011	1.833	0.005 ± 0.010	2.000	0.790*, 0.871***

a Variability represents the ratio of standard deviation to mean value.
**P*-value (patient group versus control group).
***P*-value (Parkinson's disease group with normal ceruloplasmin levels versus Parkinson's disease group with decreased ceruloplasmin levels).
****P*-value (Parkinson's disease group with normal ceruloplasmin levels versus Parkinson's disease group with decreased ceruloplasmin levels versus control group) after adjusting for age and gender.
CA = caudate; CP = ceruloplasmin; F = female; FWM = frontal white matter; GP = globus pallidus; M = male; MMSE = Mini-Mental State Examination; PD = Parkinson's disease; PU = putamen; RN = red nucleus; SN = substantia nigra; TH = thalamus; UPDRS = Unified Parkinson's Disease Rating Scale.

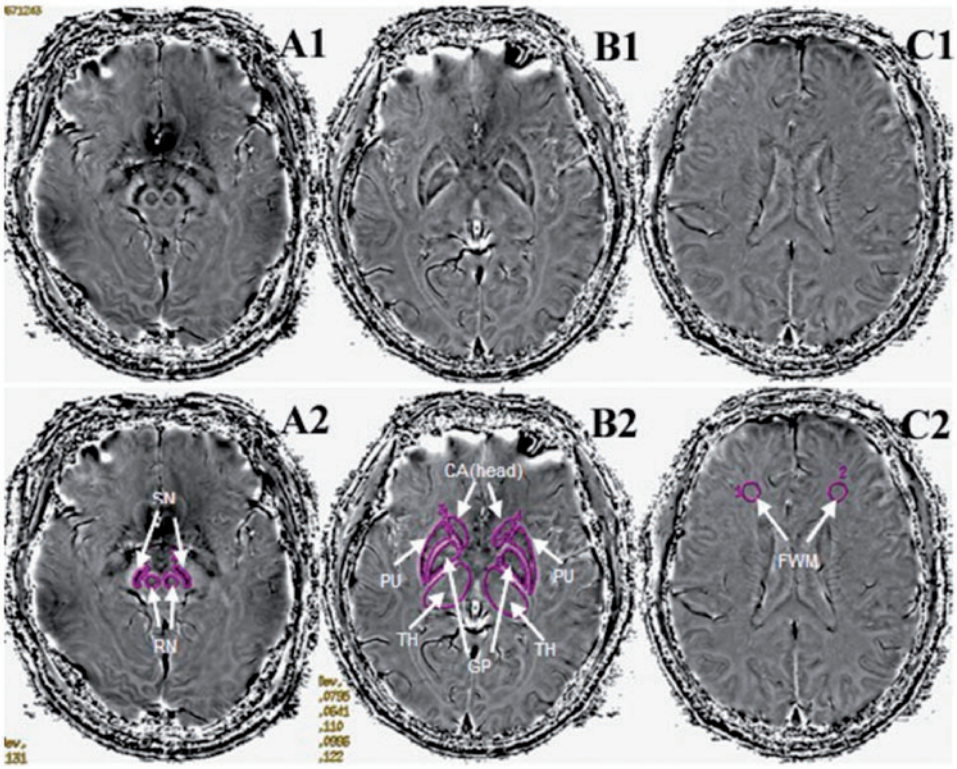

Figure 1 Illustration of the corrected phase images (**A1**, **B1** and **C1**) and the selected regions of interest (**A2**, **B2** and **C2**). CA = caudate; FWM = frontal white matter GP = globus pallidus; PU = putamen; RN = red nucleus; SN = substantia nigra; TH = thalamus.

carried out using the Statistical Package for the Social Sciences (SPSS for Windows, version 13.0; SPSS Inc., Chicago, IL, USA).

Results

Phase values of susceptibility-weighted imaging correlates with brain iron levels

To determine whether the phase values of SWI correlate with brain iron levels, the bilateral average phase values for each brain region in Parkinson's disease and control groups were measured (Table 1). We applied Pearson's correlation analysis to determine the correlation between iron concentrations in different brain regions, as previously assessed by biochemical methods (Hallgren and Sourander, 1958), and our SWI phase values. The results showed a strong inverse correlation between the SWI phase values and previously published iron concentrations (Hallgren and Sourander, 1958) in each normal adult brain region measured ($r = -0.8762$, $P = 0.010$, Fig. 2), supporting the use of SWI phase-corrected images as a viable tool for estimating regional iron content in the human brain. In addition, our study showed that extrapyramidal structures, which have significantly higher iron levels, had a lower phase value (<–0.05 rad) and a low coefficient of variation, as compared with frontal white matter and thalamus (Table 1), indicating that SWI is suitable for determining iron levels in extrapyramidal structures.

Specific change in nigral bilateral average phase values in patients with Parkinson's disease

In patients with Parkinson's disease, nigral bilateral average phase values (adjusted for gender and age) were lower compared with control subjects ($P < 0.001$). In contrast, the bilateral average phase values of all other brain regions measured did not differ significantly between patients with Parkinson's disease and control subjects (Table 1).

Correlation between serum ceruloplasmin levels, motor score and phase values

Correlation analyses of the bilateral average phase values of each brain region were performed with independent variables including age, onset age, duration of disease, UPDRS score, UPDRS motor score and serum ceruloplasmin levels. Significant correlations were only observed between nigral bilateral average phase values and serum ceruloplasmin levels ($r = 0.517$, $P < 0.001$, Fig. 3A) or UPDRS motor score ($r = -0.369$, $P = 0.013$).

Furthermore, we divided patients with Parkinson's disease into two subgroups: (i) those with lowered serum ceruloplasmin levels (serum ceruloplasmin <0.20 g/l) and those with normal serum ceruloplasmin levels (serum ceruloplasmin ≥0.20 g/l). When we

compared the bilateral average phase values of the substantia nigra in the two subgroups and the control group using the co-ANOVA analyses, we found significant differences among them ($P < 0.001$). The nigral bilateral average phase values of patients with lowered serum ceruloplasmin levels were significantly lower than those of control subjects ($P < 0.001$) and patients with normal serum ceruloplasmin levels ($P < 0.001$), whereas the bilateral average phase values in the other brain regions did not exhibit significant differences (Table 1).

To assess the relationship between disease severity and phase values, we divided patients with Parkinson's disease into three subgroups according to their Hoehn and Yahr score: 24 at stage I, 10 at stage II and 11 at stage III. By co-ANOVA analysis adjusted for age, gender and serum ceruloplasmin levels, we found significant differences between nigral bilateral average phase values in the three subgroups ($P = 0.013$) but not in other brain regions (Table 2). Furthermore, the lateralized UPDRS motor score from the clinically most affected side was correlated with the phase values of the contralateral substantia nigra in patients ($r = -0.372$, $P = 0.012$; Fig. 3B). Together these results show that the severity of Parkinson's disease, as measured by motor score, is highly correlated with the SWI phase shift.

No effect of medication on phase shift and serum ceruloplasmin levels

Among the patients with Parkinson's disease, 27 patients had previously been treated with Parkinson's disease medication and 18 patients had not. In order to probe whether medication could play a role in serum ceruloplasmin levels and phase values in different regions, we analysed our data according to whether the patient had been previously medicated and found that there were no significant differences in either serum ceruloplasmin levels (non-medicated patients: 0.191 ± 0.029 g/l; medicated patients: 0.193 ± 0.036 g/l; $P = 0.678$) or phase values in any brain region measured (Fig. 4). Importantly, in both the medicated ($r = 0.601$, $P = 0.002$) and non-medicated patient groups ($r = 0.689$, $P = 0.006$), lower ceruloplasmin levels were correlated more with nigral bilateral average phase values even after adjusting for age, gender, duration of disease and UPDRS score. In addition, we analysed the partial correlation between SWI phase value and serum ceruloplasmin levels in all patients with Parkinson's disease considering 'medicated or not' as a correction ($r = 0.618$, $P < 0.001$). The results showed that there were no significant differences between the medicated and non-medicated Parkinson's disease subgroups.

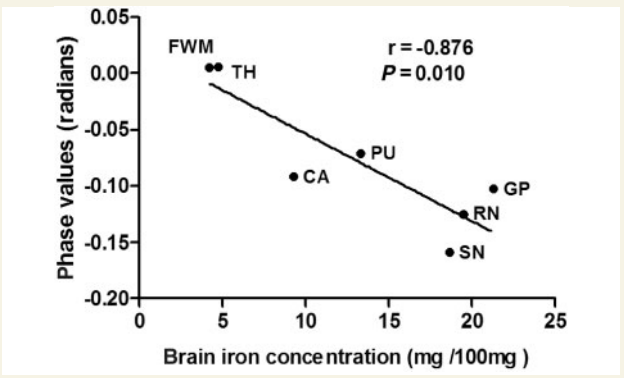

Figure 2 The correlation between bilateral average phase values and iron concentrations as published by Hallgren and Sourander (1958) in the normal adult brain. CA = caudate; FWM = frontal white matter; GP = globus pallidus; PU = putamen; RN = red nucleus; SN = substantia nigra; TH = thalamus.

Discussion

Iron deposition in the brain has been reported to be involved in the pathogenesis of many neurodegenerative disorders, especially Parkinson's disease (Zecca *et al.*, 2004; Rhodes and Ritz, 2008). Here we showed that nigral iron content, as measured using SWI, was significantly elevated in patients with Parkinson's disease compared with control subjects. Furthermore, we demonstrated that the change in the phase shift of the SWI correlated with lowered serum ceruloplasmin levels, suggesting that disrupted

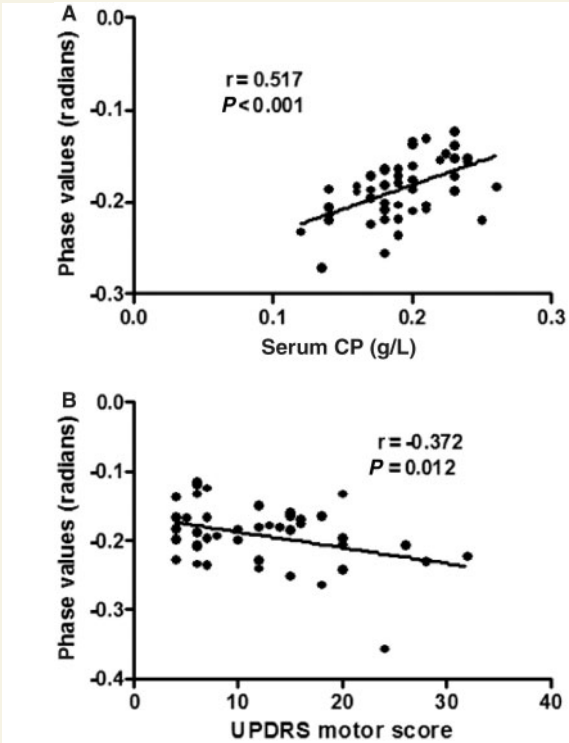

Figure 3 Correlations between nigral phase values and the UPDRS motor score or serum ceruloplasmin levels. (**A**) Correlation between bilateral average phase values of substantia nigra and serum ceruloplasmin (CP) levels in patients with Parkinson's disease. (**B**) Correlation between the phase values of contralateral substantia nigra and the lateralized UPDRS motor score from the clinically most affected side in patients with Parkinson's disease.

Table 2 The correlations between bilateral average phase values in each brain region and disease severity

	Hoehn and Yahr stage I ($n=24$)	Hoehn and Yahr stage II ($n=10$)	Hoehn and Yahr stage III ($n=11$)	*P*-value
Age	57.21 ± 8.60	53.90 ± 13.96	56.55 ± 12.87	0.789
Serum ceruloplasmin (g/l)	0.196 ± 0.031	0.183 ± 0.029	0.190 ± 0.040	0.697
Bilateral average phase values (radians)				
SN	−0.177 ± 0.025	−0.183 ± 0.034	−0.208 ± 0.041	0.013
RN	−0.143 ± 0.044	−0.134 ± 0.039	−0.124 ± 0.061	0.653
PU	−0.079 ± 0.041	−0.053 ± 0.019	−0.071 ± 0.040	0.253
GP	−0.106 ± 0.016	−0.113 ± 0.050	−0.113 ± 0.052	0.876
CA (head)	−0.099 ± 0.035	−0.083 ± 0.026	−0.103 ± 0.035	0.252

CA = caudate; GP = globus pallidus; PU = putamen; RN = red nucleus; SN = substantia nigra.

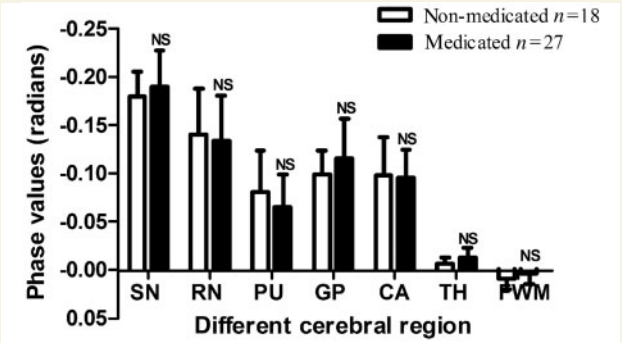

Figure 4 Bilateral average phase values (in radians) of medicated and non-medicated patients with Parkinson's disease. CA = caudate; FWM = frontal white matter GP = globus pallidus; NS = not significant; PU = putamen; RN = red nucleus; SN = substantia nigra; TH = thalamus.

ceruloplasmin metabolism may be an important event in the pathogenesis of Parkinson's disease.

Consistent with previous studies (Xu *et al*., 2008), we found a strong and statistically significant negative correlation between SWI phase values and measured iron concentrations (Hallgren and Sourander, 1958) in all analysed regions of the normal adult brain ($r=-0.876$, $P=0.010$, Fig. 2), supporting use of SWI phase-corrected imaging as a viable tool for estimating regional iron content. We acknowledge that a direct relationship between iron mass and SWI phase has only previously been established for point sources of iron (McAuley *et al.*, 2010). However, given the statistically significant correlations between SWI phase values and measured brain iron concentrations, we believe that it is reasonable to use SWI phase values as a relative measure of brain iron concentration. Furthermore, in our analyses, care was taken to avoid regions clearly affected by the dipole effect when drawing regions of interest.

Compared with control subjects, we found that patients with Parkinson's disease exhibited significantly lower bilateral average phase values, indicating enhancement of iron content in the substantia nigra. No significant differences were found in other brain regions examined. As an oxidative stress-prone structure, due to its enrichment of dopaminergic neurons, the substantia nigra may be more vulnerable to iron deposition than other brain regions. By subgroup analysis, we found that nigral phase values in patients with Parkinson's disease were correlated with disease severity. The phase values of substantia nigra in patients in the advanced stages of Parkinson's disease were lower than substantia nigra values in early-stage Parkinson's disease patients ($P=0.013$, Table 2). This conclusion is also supported by the result that the lateralized UPDRS motor score from the clinically most affected side was significantly associated with phase values of the contralateral substantia nigra in patients with Parkinson's disease. By the analysis of medicated and non-medicated patients, we found that medication did not influence serum ceruloplasmin levels nor iron concentration in different brain regions.

Importantly, we found that nigral bilateral average phase values in patients with Parkinson's disease with decreased serum ceruloplasmin levels were significantly lower than in patients with normal serum ceruloplasmin levels and in control subjects, whereas nigral bilateral average phase values in patients with normal serum ceruloplasmin levels did not significantly differ from control subjects (Table 1). Ceruloplasmin has been demonstrated to antagonize oxidative damage in the central nervous system (Patel *et al*., 2002). Decreased serum ceruloplasmin levels might reflect the downregulation of ceruloplasmin synthesis in the brain, which could lead to more severe oxidative stress in the substantia nigra in patients with Parkinson's disease.

Our results suggest that Parkinson's disease is a heterogeneous disorder that undergoes different pathophysiological alterations and can be at least divided into two subgroups: (i) patients with decreased serum ceruloplasmin levels exhibiting increased iron deposition in the substantia nigra; and (ii) patients with normal serum ceruloplasmin levels exhibiting nigral iron content similar to control subjects.

Our current study provides the possibility of magnetic resonance based susceptibility-weighted imaging as a tool for the diagnosis and assessment Parkinson's disease. Nigral dopaminergic neurons have already degenerated over 50% when the first movement symptoms of the disease appear (Hornykiewice, 1998), which is too late to implement neuroprotective strategies. For effective neuroprotective and disease-modifying treatment, it is essential to find risk factors and/or biomarkers involved in specific

pathophysiological alterations that enable the identification of subjects at risk (Berg and Hochstrasser, 2006). Serum ceruloplasmin, as an abundant glycoprotein, can be determined cheaply and conveniently for clinical screening. By combining serum ceruloplasmin detection with measurement of brain iron using magnetic resonance imaging, we can differentiate patients with Parkinson's disease into subsets of cases associated with nigral iron deposition from those without iron deposition. Furthermore, using these methods, we could identify subjects at higher risk of developing Parkinson's disease prior to the manifestation of symptoms. These methods may also prove to be significant, both in the validation of neuroprotective agents that prevent the progression of early-stage Parkinson's disease and for the development of new drugs such as iron-chelating treatments in the future. Thus, combining measurements of serum ceruloplasmin levels and SWI phase values will probably be important in many aspects of the diagnosis and assessment of Parkinson's disease.

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Supplementary material

Supplementary material is available at *Brain* online.

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