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Quantitative susceptibility mapping as an indicator of subcortical and limbic iron abnormality in Parkinson's disease with dementia

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ABSTRACT

Late stage Parkinson's disease (PD) patients were commonly observed with other non-motor comorbidities such as dementia and psychosis. While abnormal iron level in the substantia nigra was clinically accepted as a biomarker of PD, it was also suggested that the increased iron deposition could impair other brain regions and induce non-motor symptoms. A new Magnetic Resonance Imaging (MRI) called Quantitative Susceptibility Mapping (QSM) has been found to measure iron concentration in the grey matter reliably. In this study, we investigated iron level of different subcortical and limbic structures of Parkinson's disease (PD) patients with and without dementia by QSM.

QSM and volumetric analysis by MRI were performed in 10 PD dementia (PDD) patients (73 ± 6 years), 31 PD patients (63 ± 8 years) and 27 healthy controls (62 ± 7 years). No significant differences were observed in the L-Dopa equivalent dosage for the two PD groups ($p = 0.125$).

Putative iron content was evaluated in different subcortical and limbic structures of the three groups, as well as its relationship with cognitive performance. One-way ANCOVA with FDR adjustment at level of 0.05, adjusted for age and gender, showed significant group differences for left and right hippocampus ($p = 0.015$ & 0.032, respectively, BH-corrected for multiple ROIs) and right thalamus ($p = 0.032$, BH-corrected). Post-hoc test with Bonferroni's correction suggested higher magnetic susceptibility in PDD patients than healthy controls in the left and right hippocampus ($p = 0.001$ & 0.047, respectively, Bonferroni's corrected), while PD patients had higher magnetic susceptibility than the healthy controls in right hippocampus and right thalamus ($p = 0.006$ & 0.005, respectively, Bonferroni's corrected). PDD patients also had higher susceptibility than the non-demented PD patients in left hippocampus ($p = 0.046$, Bonferroni's corrected). The magnetic susceptibilities of the left and right hippocampus were negatively correlated with the Mini-Mental State Examination score ($r = -0.329$ & -0.386, respectively; $p < 0.05$).

This study provides support for iron accumulation in limbic structures of PDD and PD patients and its correlation with cognitive performance, however, its putative involvement in development of non-motor cognitive dysfunction in PD pathogenesis remains to be elucidated.

1. Introduction

Patients with Parkinson's disease (PD) in advanced stage may develop concomitant non-motor symptoms related to neuropsychiatric or cognitive disturbances (Tolosa et al., 2014), significantly exacerbating their disability. One of the most common non-motor

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manifestations in Parkinson's disease is dementia (PDD), for which the prevalence and annual incidence are estimated at respectively 30–40% (Burn and Yarnall, 2014; Emre et al., 2007; Goetz et al., 2008) and 10% (Goetz et al., 2008) of PD patients. This indicates that such potentially disabling complication of PD should be carefully addressed in clinical management. Patients with PDD generally experience significant cognitive decline, distinct from that due to Alzheimer's disease (AD), including impairment of executive function, attention, visuospatial function, constructional praxis, and memory (Burn and Yarnall, 2014; Goetz et al., 2008). Notably, greater deficits in executive and visuospatial functions and lesser in language functions are observed in PDD than AD (Burn and Yarnall, 2014; Goetz et al., 2008). While some studies propose an association between Apolipoprotein E (APOE) and the dementia symptoms of PDD (Irwin et al., 2012; Monsell et al., 2014), others do not support such claims (Burn and Yarnall, 2014; Mengel et al., 2016), thus rendering the mechanism underlying development of dementia in PD and its relationship with the AD-type dementia to be inconclusive.

Various studies have been performed to identify abnormalities in the brain of PDD patients. Imaging studies such as Magnetic Resonance Imaging (MRI) reported structural change in the hippocampus and amygdala of PDD patients, suggesting that the dementia symptom in PD might be associated with limbic atrophy (Aarsland et al., 2008); one study by Kalaitzakis et al. also suggested the involvement of limbic system in PDD, with the association between dementia and α-synuclein pathology in the limbic structures being observed (Kalaitzakis et al., 2009). These findings suggest plausible association between limbic abnormality and the dementia symptom, and that further investigation of the involvement of limbic structures in PDD is warranted.

Abnormal subcortical iron deposition in PD has been postulated to be the cause of the degeneration of dopaminergic neurons in the substantia nigra pars compacta (Hare et al., 2013; Jenner, 1991; Rouault and Cooperman, 2006). In a hypothesis of PD neurodegeneration, iron is suggested to induce α-synuclein pathology (Hare et al., 2013). α-synuclein is a presynaptic neuronal protein that is abundant in human brain and can be found in different brain regions including neocortex, hippocampus, substantia nigra, thalamus, and cerebellum. Abnormal aggregation of α-synuclein in PD patients, a clinical pathological hallmark of the disease, contributes to the formation of Lewy bodies which the major component is α-synuclein (Marques and Outeiro, 2012; Stefanis, 2012). Such pathological feature rendered PD to be considered as a type of synucleinopathies. Since α-synuclein aggregation has also been observed in limbic structures and is itself associated with the onset of dementia in PDD, we hypothesize that iron accumulation in PD could also be involved in the later overt development of neuropsychiatric symptoms in PD patients.

Quantitative Susceptibility Mapping (QSM) is a novel technique which allows the determination of tissue's bulk magnetic susceptibility distribution from gradient echo magnetic resonance phase images. A previous study by Langkammer et al. reported a strong linear correlation between chemically determined iron concentration and bulk magnetic susceptibility in grey matter structures (Langkammer et al., 2012). The objective of this study is to identify the role of abnormal iron metabolism in PD-type dementia by comparing the in vivo putative iron content measured with QSM, of different subcortical and limbic brain structures amongst healthy subjects and PD patients with or without dementia. In addition, any association between iron and the expression of dementia and psychotic symptomatology in PD is addressed. MR volumetric analysis of the subcortical and limbic brain structures is also performed.

2. Materials and methods

2.1. Participants

This study was approved by the local Institutional Review Board. The recruited participants were divided into three groups based on clinical diagnosis and result of neuropsychiatric assessments. A total of 68 participants were recruited with referral from experienced physicians. All participants or their caregivers were carefully explained for the study by the responsible medical officers before full written informed consent was obtained. The study cohort comprised of 10 PDD patients (8 males, mean age ± S.D. = 73 ± 6 years, mean illness duration ± S.D. = 13 ± 8 years), 31 non-demented PD patients (17 males, mean age ± S.D. = 63 ± 8 years, mean illness duration ± S.D. = 8 ± 5 years) and 27 healthy controls (14 males, mean age ± S.D. = 62 ± 7 years). PD was diagnosed using the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria (Hughes et al., 1992). PD patients were classified as PDD when they fulfilled the level one diagnostic criteria proposed by the Movement Disorder Society (MDS) (Dubois et al., 2007; Holden et al., 2016; Vasconcellos and Pereira, 2015). Patients with other known neurodegenerative disorders were excluded. Severity of motor deficit was assessed with both Section III of Unified Parkinson's Disease Rating Scale (UPDRS-III) and Hoehn & Yahr staging (Hoehn and Yahr, 1967). The cumulative L-Dopa Equivalence (mg) of the PD patients were also obtained for the evaluation of the differences between the study cohorts.

2.2. Neuropsychiatric assessments

Neuropsychiatric status of all subjects were assessed with the following tests: Mini-mental State Examination (MMSE) to assess cognitive impairment (Folstein et al., 1975); Parkinson Psychosis Rating Scale (PPRS) and Positive and Negative Syndrome Scale (PANSS) to examine the severity of psychosis (Friedberg et al., 1998); Self-assessment of Montgomery-Åsberg Depression Rating Scale (MADRS-S) to measure the severity of depression symptom (Kay et al., 1987; Montgomery and Asberg, 1979), and Yale-Brown Obsessive Compulsive Scale (Y-BOCS) to measure obsessive-compulsive symptoms (Goodman et al., 1989). The complete demographics and the clinical neuropsychiatric profile of all subjects are listed in Table 1.

Table 1
Demographics and results of neuropsychiatric assessment of healthy controls, non-demented PD and PDD patients.

	Healthy	PD	PDD	p-value
N	27	31	10	–
Age (years)	62.0 ± 7.0	63.1 ± 8.3	72.6 ± 5.8	0.001*
Gender (M:F)	14:13	17:14	8:2	0.29
Duration of illness (years)	n/a	7.6 ± 4.6	12.8 ± 8.1	0.08
Hoehn & Yahr Stage	n/a	2.7 ± 0.8	2.7 ± 1.2	0.90
UPDRS-III	n/a	17.4 ± 9.8	33.7 ± 15.7	< 0.001**
MMSE	28.8 ± 1.0	28.3 ± 1.7	19.3 ± 5.0	< 0.001**
PPRS	6.1 ± 0.3	6.6 ± 0.9	9.8 ± 2.9	< 0.001**
PANSS	30.9 ± 3.3	38.2 ± 8.3	52.9 ± 15.6	< 0.001**
MADRS-S	0.4 ± 1.1	2.4 ± 2.8	3.9 ± 3.0	< 0.001**
Y-BOCS	0.0 ± 0.2	0.9 ± 2.4	3.2 ± 6.3	0.016*
L-Dopa Equivalence (mg)	n/a	663.2 ± 416.8	1174.4 ± 935.6	0.125

Note: Values on the table are displayed as mean ± S.D. UPDRS-III: Section III (motor examination) of the Unified Parkinson's Disease Rating Scale. MMSE: Mini-Mental State Examination; PPRS: Parkinson's Psychosis Rating Scale; PANSS: Positive and Negative Syndrome Scale; MADRS-S: Self-assessment of the Montgomery-Åsberg Depression Rating Scale; Y-BOCS: Yale-Brown Obsessive Compulsive Scale.

* $p < 0.05$

** $p < 0.001$.

2.3. Image acquisition

MRI was performed with a Philips Achieva 3.0 T MRI Scanner with an 8-channel SENSE head coil for reception. Whole brain sagittal T1-weighted images were acquired with the 3D T1-TFE sequence with the following parameters: TE/TR = 3.2/7.0 ms, flip angle = 8°, FOV = 250 × 250 × 155 mm^3, matrix size = 256 × 256 × 155, nominal and reconstructed resolution = 0.97 × 0.97 × 1 mm^3, number of excitations (NEX) = 1. Axial susceptibility weighted images (SWI) images were acquired with a velocity-compensated 3D fast-field echo sequence with the following parameters: TE/TR = 23/28 ms, flip angle = 15°, FOV = 230 × 230 × 180 mm^3, acquisition matrix size = 256 × 256 × 180, nominal resolution = 0.9 × 0.9 × 1 mm^3, reconstructed resolution = 0.45 × 0.45 × 1 mm^3, NEX = 1.

2.4. Image post-processing

QSM computations were performed using MATLAB R2014a. The phase image was unwrapped with the Laplacian-based algorithm (Li et al., 2011; Schofield and Zhi, 2003) and was further processed for background phase removal with RE-SHARP (Sun and Wilman, 2014). QSM dipole inversion was performed with the L1-norm total-variation-based regularization algorithm with magnitude image as structural prior (Bilgic et al., 2014; Bilgic et al., 2012).

Delineation of subcortical and limbic structures was performed with FIRST in FSL. FIRST was applied to the affine-registered T1-TFE image for the segmentation of bilateral caudate nuclei, putamen, globus pallidus, amygdala, hippocampi, and thalamus (Fig. 1), which were subsequently converted into binary masks for the region-of-interest (ROI) analysis of regional magnetic susceptibility and regional volume. Bilateral substantia nigra, red nuclei and dentate nuclei were manually defined (by DTHL supervised by a neuroradiologist, HKFM) on the average magnitude image of the control group in the MNI152 standard space. CSF of the lateral ventricle was segmented for the normalization purpose. This was performed by the segmentation of all CSF using FSL. An ROI which identified the slices of the lateral ventricle was delineated for each subject and was subsequently applied to the CSF mask to segment the CSF of the lateral ventricle. This ROI was eroded by 1.5 mm to avoid partial volume effect. The delineated structures were converted into binary masks, which were then inversely warped back to the subject's native space for ROI analysis.

2.5. Data analysis

Group differences in age and neuropsychiatric assessments were examined by one-way ANOVA and post-hoc test with Bonferroni's correction, and gender composition was tested by Pearson's Chi-Square test. Comparison of motor symptom between the two PD groups was assessed by independent sample *t*-test. Measurement of subcortical and limbic magnetic susceptibility was performed in the subject's native space by applying the ROIs on the QSM images. The measured susceptibility value of each structure was adjusted by normalizing the relative magnetic permeability of the structures to that of CSF in the lateral ventricle. The normalized structural susceptibility was compared between groups. The ROIs were also used to calculate the gross volumes of the subcortical and limbic structures, and the measured volumes were normalized to that of the whole brain volume for each subject before statistical comparison. All comparisons were performed with the SPSS Statistics Package v.22 (IBM Corporation, New York, U.S.) at the significance level of $p < 0.05$. One-way ANCOVA and post-hoc test with Bonferroni's correction were employed for multiple group comparison. Age and gender were considered as confounding factors and were adjusted in the analysis. For each significant result observed, the assumption of linear relationship between covariates and the measured susceptibility was evaluated. Group interaction effect between the covariates and the normalized structural susceptibility was incorporated into the ANCOVA model to examine the effect of covariates on the measured susceptibility. To account for the statistical analyses of multiple ROIs in this study, Benjamini-Hochberg procedure (BH) was employed to control the false discovery rate (FDR) at the level of 0.05.

Correlation between equivalent levodopa dosage and subcortical or limbic structure volume of the two PD groups was examined by

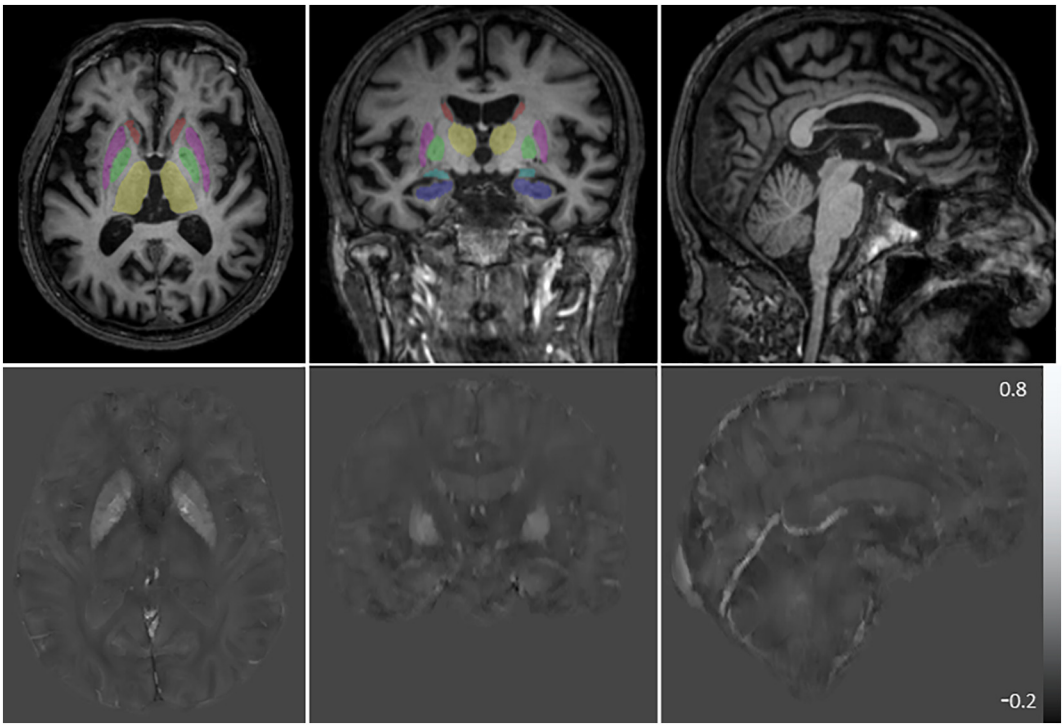

Fig. 1. (Upper row) Affine registered 3D-T1-TFE image of one of the PDD patients overlaid with the segmented subcortical and limbic structures. The segmentation is performed with FIRST. (Lower row) The corresponding L1-regularized QSM images in the three orthogonal planes.

Pearson's correlation test to assess the effect of medication on brain atrophy. Correlation between structural magnetic susceptibility and psychiatric status of the subjects, represented by the neuropsychiatric scores, was examined with non-parametric Spearman's rank correlation test. Effects of age and gender were adjusted in the analyses.

3. Results

3.1. Demographics and neuropsychiatric assessment

Demographics and results of the neuropsychiatric assessments are tabulated in Table 1. Significant difference existed between the mean ages of the three groups. Post-hoc test showed that mean age of PDD patients was significantly higher than the other two groups ($p < 0.01$). No significant difference was observed in gender composition ($p = 0.29$). Differences in motor symptom scores and duration of illness between the two PD groups were examined. While the two PD groups showed similar duration of illness ($p = 0.08$) and similar PD stage by Hoehn & Yahr staging ($p = 0.90$), the UPDRS-III of PDD patients was found to be significantly higher than that of the non-demented PD patients ($p < 0.001$). No significant differences were observed in the L-Dopa equivalent dosage for the two PD groups ($p = 0.125$).

Neuropsychiatric assessment results were compared with one-way ANOVA, and significant differences between the three groups were observed in all tests. Post-hoc test suggested that PDD patients had significantly worse scores than the other two groups in the PPRS, PANSS and MMSE assessments ($p < 0.001$, all), and worse than the healthy controls for MADRS-S ($p = 0.006$) and Y-BOCS ($p = 0.012$).

3.2. Iron loading by measuring magnetic susceptibility

Average magnetic susceptibility measured for each subcortical and limbic structure was compared between the three groups, and the results are summarized in Figs. 2 and 3, with Fig. 2 indicating the result of one-way ANCOVA while Fig. 3 showing the results of the post-hoc test. One-way ANCOVA at the FDR level of 0.05, adjusted for age and gender, showed significant group differences for left and right hippocampus ($p = 0.015$ & 0.032, respectively, BH corrected) and right thalamus ($p = 0.032$, BH corrected). Post-hoc test with multiple comparison correction suggested higher magnetic susceptibility in PDD patients than healthy controls in the left and right hippocampus ($p = 0.001$ & 0.047, respectively, Bonferroni's corrected). PDD patients also had higher susceptibility than the non-demented PD patients in left hippocampus ($p = 0.046$, Bonferroni's corrected). Significance observed for the right thalamus was due to the significant difference between non-demented PD patients and healthy controls ($p = 0.005$, Bonferroni's corrected) after post-hoc test. The PD patients were also found to had higher magnetic susceptibility than the healthy controls in the right hippocampus ($p = 0.006$, Bonferroni's corrected).

The linear relationship between age and measured susceptibility of bilateral hippocampus and right thalamus was evaluated with linear regression. Susceptibility of bilateral hippocampus showed a moderate correlation with age (left hippocampus: $r = 0.384$, $p = 0.001$; right hippocampus: $r = 0.396$, $p = 0.001$). For right thalamus, insignificant linear correlation was observed ($r = 0.026$, $p = 0.836$). Group interaction effects between age and the measured susceptibility of the three brain structures were examined and result of all three regions were found to be insignificant (left hippocampus: $p = 0.476$; right hippocampus: $p = 0.494$; right thalamus: $p = 0.354$).

3.3. Comparison of the volume of subcortical and limbic structures

The average normalized volumes of the subcortical and limbic structures were generally smaller in the PDD patients, yet no statistical significance was observed for all subcortical and limbic structures after FDR adjustment with Benjamini-Hochberg procedure. Correlation analysis showed mild positive correlation between levodopa dosage and normalized volume of left substantia nigra in the two PD groups, with Pearson's coefficient of 0.333 ($p = 0.041$). Similar result also observed in left and right dentate nucleus, with Pearson's coefficient of 0.345 ($p = 0.034$) and 0.336 ($p = 0.039$), respectively.

3.4. Enlargement of lateral ventricle

The size of the lateral ventricle was estimated and compared between the three groups and the general trend showed that the size increased with disease progression (Fig. 4). One-way ANCOVA showed significant group differences ($p = 0.031$). Post-hoc test suggested that size of lateral ventricle was significantly larger in PDD patients than healthy controls ($p = 0.027$) while no difference was found between the non-demented PD patients and healthy controls.

3.5. Neuropsychiatric correlation

There were significant correlations between some of the neuropsychiatric scores and bilateral thalamus and hippocampus (Fig. 5). Bilateral thalamus showed mild positive correlation with PPRS (Correlation coefficient for left thalamus: 0.251, $p = 0.039$; for right thalamus: 0.247, $p = 0.043$), while bilateral hippocampus displayed moderate positive correlation with PPRS (Correlation coefficient for left hippocampus: 0.259, $p = 0.033$; for right hippocampus: 0.306, $p = 0.011$) and PANSS (Correlation coefficient for left hippocampus: 0.279, $p = 0.021$; for right hippocampus: 0.406, $p = 0.001$), and moderate negative correlation with MMSE (Correlation coefficient for left hippocampus: -0.329, $p = 0.006$; for right hippocampus: -0.386, $p = 0.001$).

4. Discussion

Post-mortem and imaging studies pointed to abnormal iron deposition in the substantia nigra pars compacta of PD patients (Bartzokis et al., 1999; Graham et al., 2000; Griffiths et al., 1999; Hare et al., 2013; Jenner, 1991; Jin et al., 2012; Kosta et al., 2006; Rouault and Cooperman, 2006; Sofic et al., 1988), but the role of iron accumulation in PD and its involvement in the later overt development of neuropsychiatric symptoms in PDD remain elusive. In current study, we found that: firstly, increased iron concentration was observed in bilateral hippocampus of PDD patients, and right hippocampus and right thalamus of PD patients, as compared to healthy controls; secondly, the iron content of bilateral hippocampus was found to be moderately correlated with both cognitive function and psychotic symptoms of PD patients.

Our current finding of hippocampal iron accumulation and its correlation with psychotic features in PDD patients should be interpreted in the light of current research.

As a cross-sectional imaging study, causation cannot be inferred and it remains unclear if the increased iron level in the hippocampus of the PDD patients is involved in the pathogenesis or purely reflects an epiphenomenon of advancing neurodegeneration of PD patients and this warrants further investigation of the causality by a longitudinal study. Although a few previous studies also reported association of hippocampal iron with memory test performance in ageing (Rodrigue et al., 2013) and Alzheimer's disease (Ding et al., 2009), no causality between hippocampal iron accumulation and the development of dementia symptom in the PDD patients could be inferred.

Our finding of hippocampal involvement in PDD concurred with a few prior studies, but nevertheless, the role of hippocampus remains controversial. Several studies have reported the association between the limbic structures and the onset of cognitive decline or dementia in PD patients (Aarsland et al., 2008; Kalaitzakis et al., 2009). Previous studies of the similar cohort (by our team) using diffusion tensor imaging (DTI) and BOLD fMRI reported that the psychotic symptoms of visual

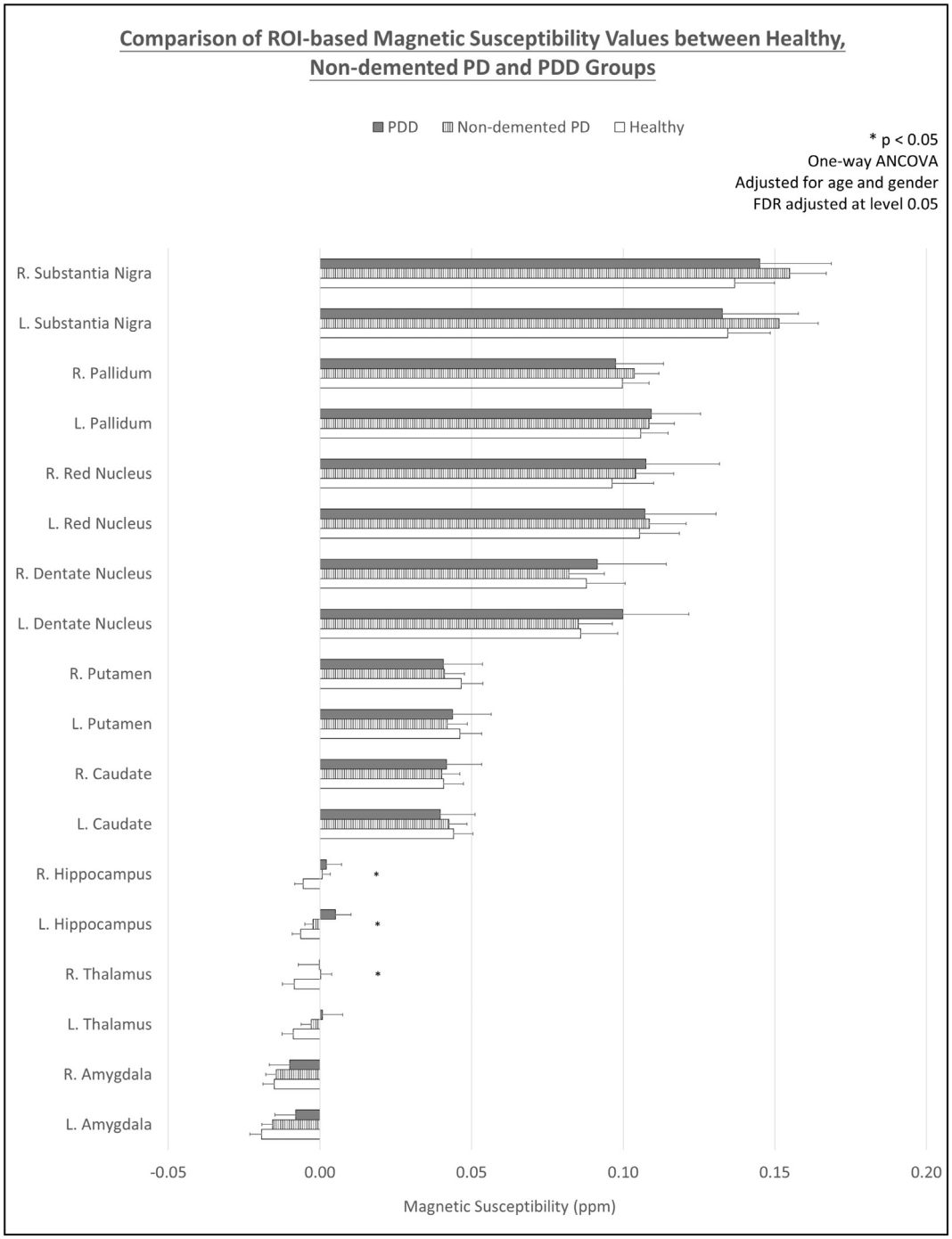

Fig. 2. Result of one-way ANCOVA comparing the magnetic susceptibility of different subcortical and limbic structures between healthy, PD and PDD groups, with FDR adjusted at level of 0.05.

hallucination in PD patients is related to the disruption of default mode network (DMN), particularly the functional connection between hippocampus with DMN and frontal region (Yao et al., 2016; Yao et al., 2014), suggesting that pathological changes to hippocampus is associated with the development of psychotic disorder in PD.

However, besides mesolimbic and mesocortical dopaminergic pathways being involved in cognitive functioning, a number of non-dopaminergic transmitter systems are affected in PD (Aarsland et al., 2017). A Positron Emission Tomography study (Klein et al., 2010) using multiple radioactive ligands found that dementia and psychosis (visual hallucination) in both Dementia with Lewy Bodies (DLB) and PDD were related to cholinergic defects and parieto-occipital hypometabolism respectively. Another Single Photon Emission Computed Tomography study (Mori et al., 2006) using ^{99m}Tc-HMPAO similarly found that visual hallucinations in DLB could be normalized with cholinesterase inhibitors due to increased perfusion in posterior visual association cortex.

The thalamus is known to function as a hub for information transfer between cerebral cortex and basal ganglia, the structure is highly involved in regulation of different motor and non-motor functions. In the current study, higher iron content is observed in the unilateral thalamus in the non-demented PD patients, which a similar result has been reported in a previous study (Langkammer et al., 2016). Other studies also suggested possible structural abnormalities in thalamus of PD

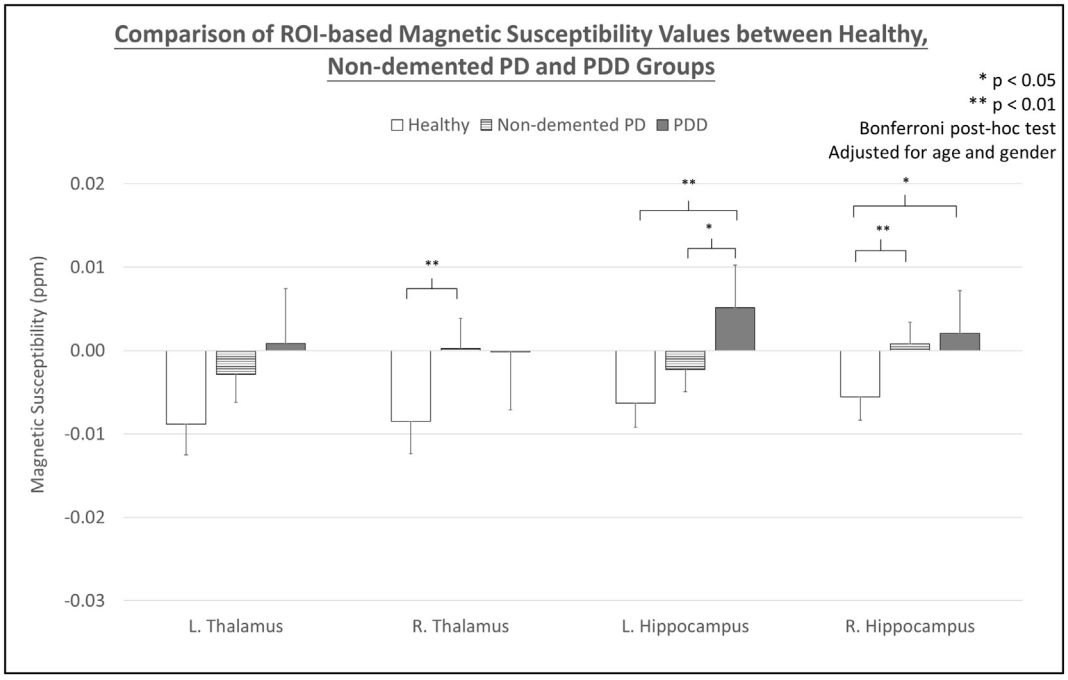

Fig. 3. Post-hoc test with Bonferroni's correction accounts for multiple comparison between the three groups. Only structures with statistical significance after one-way ANCOVA adjusted for FDR are considered for the post-hoc test. Data for left thalamus is included as a reference to that of right thalamus.

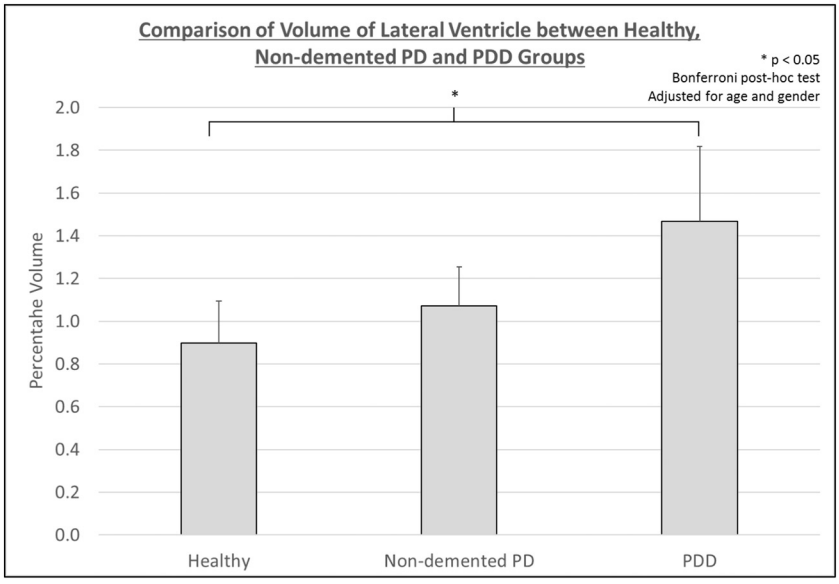

Fig. 4. Comparison of the estimated volume of lateral ventricle between healthy, non-demented PD and PDD groups.

patients (Danti et al., 2015; Deng et al., 2016; Gerrits et al., 2016). This study suggests the plausible involvement of thalamus in development of PD's motor symptoms with the deposition of iron in the structures. While the PDD group does not show significance after post-hoc test in this study, this could be possibly explained by the small sample size of the group.

We observed significant enlargement of the lateral ventricle of PDD patients vs healthy controls, which has been commonly observed and reported in AD-type dementia (Förstl et al., 1995; Luxenberg et al., 1987), and PD patients with mild cognitive impairment (MCI) (Dalaker et al., 2011). These suggest that cognitive impairment in neurodegenerative disease is closely related to the underlying brain atrophy as reflected by enlargement of lateral ventricle.

The effect of medication, i.e. prescription of levodopa for treatment of PD, and its relationship with the result of this study is uncertain. We assessed the correlation of levodopa dose with normalized volume of different brain region and controlled for age and gender of the patients. It was observed that left substantia nigra and bilateral dentate nucleus showed positive correlation with levodopa dosage, suggesting possible effect of levodopa in preserving the deep brain grey matter nuclei in the PD patients.

The effect of age on the brain iron accumulation of healthy subjects, as well as PD patients, has been previously demonstrated (Acosta-Cabronero et al., 2016; Acosta-Cabronero et al., 2017). While no significant association between age and hippocampal iron content was observed in healthy subjects (Acosta-Cabronero et al., 2016), moderate age-dependent effect of hippocampal iron was reported in PD patients (Acosta-Cabronero et al., 2017). To account for the effect of age, one-

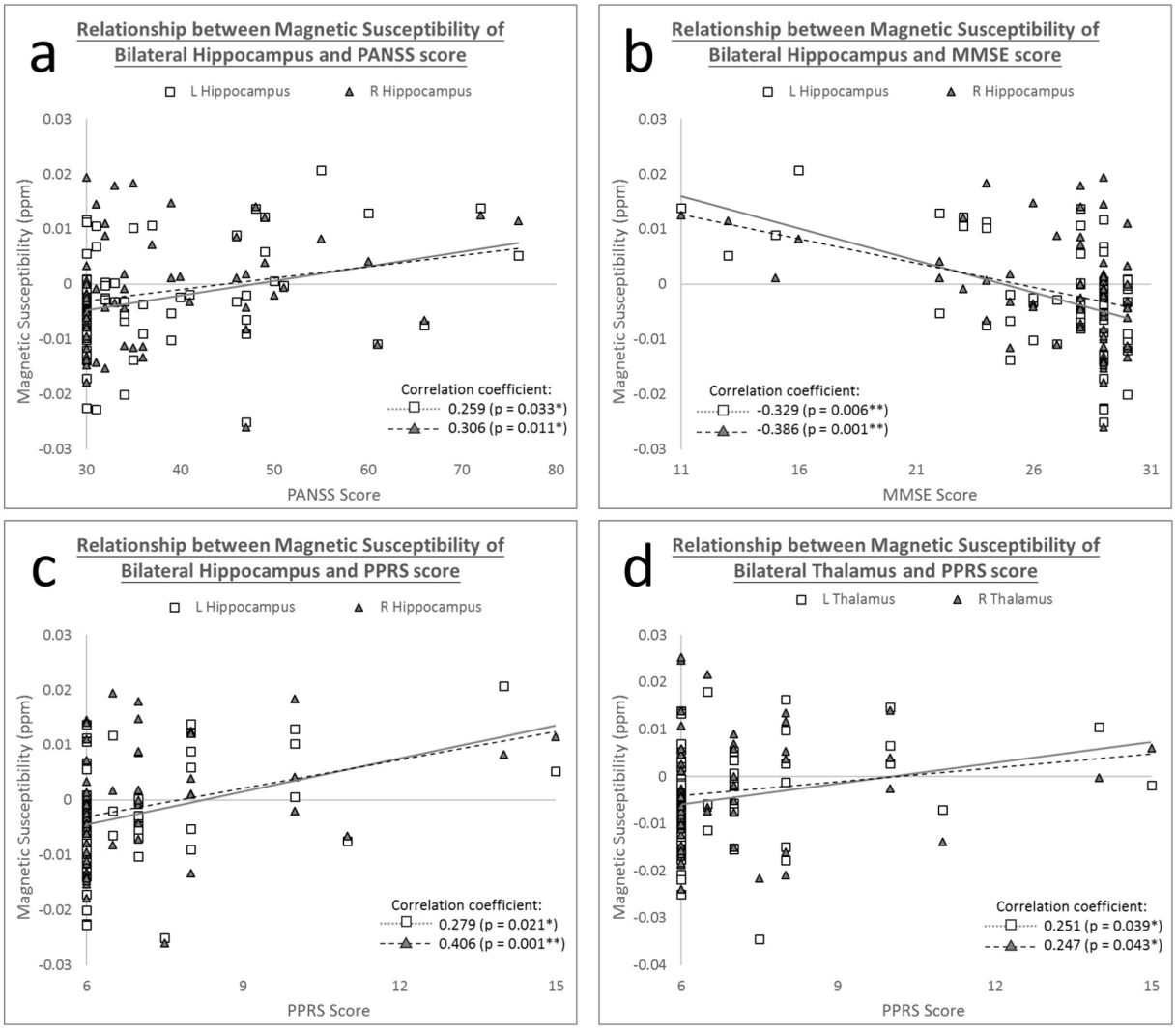

Fig. 5. Scatter plots showing the relationship between the measured susceptibility of bilateral hippocampus and (a) PANSS score, (b) MMSE score, (c) PPRS score; (d) bilateral thalamus and PPRS score.

way ANCOVA with age as a covariate was performed. The effect of age on the measured magnetic susceptibility of hippocampus was further examined by linear regression of age and measured susceptibility and incorporating the group interaction of the two terms into the ANCOVA model. The moderate correlation between age and measured susceptibility of hippocampus supported the use of ANCOVA to adjust the effect of age. It was also demonstrated that interaction effect was not significant in the statistical model adopted in this study, and age had the same effect on the measured susceptibilities in all three groups. This result supported the claim that the differences of hippocampal iron observed in this study shall be mainly due to the differences between groups.

One of the major limitations of our current study is the sample size of the PDD group. In this study, the PDD group consisted of 10 patients only, as opposed to 27 healthy controls and 31 non-demented PD patients. However, it should be noted that the PDD group is difficult to recruit due to the very stringent inclusion and exclusion criteria of the group. Since the current study is cross-sectional in design, the correlation of increased iron level in the hippocampus of the PDD patients and the neuropsychiatric scores could be non-specific and due to parallel association, both features simply reflecting advanced disease state. A longitudinal study is warranted to further investigate the role of iron in the pathogenesis of advancing neurodegeneration of PD patients and its plausible causality to the memory decline in PDD. In this study, we examined simultaneously quite a number of subcortical and limbic structures in each evaluation. To account for the problem of multiple statistical tests and concerned with the statistical power in the correction, FDR with Benjamini-Hochberg Procedure was adopted to keep the false discovery rate of each evaluation to significance level of 0.05. Another limitation of this study is concerned with the neuropsychiatric presentation of PD patients. Previous study suggests that demented PD patients usually exhibit other non-motor comorbidities, while over 50% of these patients might have visual hallucination or other psychotic symptoms (Burn and Yarnall, 2014). It would have been ideal to have recruited patients with PDD without psychosis; however, the high comorbidity of psychosis in late PD suggests that the former is part and parcel of disease progression. It is understood that psychotic features in PDD might be related to visuospatial impairment and the visual cortex shall be one of the region of interest for the investigation of non-motor symptoms in PDD. In this study, however, we did not measure posterior and occipital cortical iron load of the patients due to the uncertainty in measuring magnetic susceptibility at the cerebral cortex. Finally, one should also note that MMSE is only a screening test of cognitive dysfunction, it is not ideal for assessment of memory decline in the clinical sense.

5. Conclusion

In our current study by QSM, higher iron deposition is observed in the bilateral hippocampus of the PDD patients as compared to healthy controls. Higher iron deposition is also observed in the unilateral hippocampus of the PDD patients when compared to non-demented PD patients. Moderate correlation of iron content in the PD and PDD patients with both cognitive and other neuropsychiatric impairment is found. Our results might provide insight on the relation between the effect of iron deposition in Parkinson's disease and cognitive dysfunction. Future longitudinal study for evaluation of the role of limbic iron concentration by QSM in PD pathogenesis, its relationship to dementia and psychosis, and potential as a clinical biomarker in monitoring treatment by iron chelation therapy in clinical trials is awaited.

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