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Is hyperhomocysteinemia associated with the structural changes of the substantia nigra in Parkinson's disease? A two-year follow-up study

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ABSTRACT

Objective: It was recently found that structural changes in the substantia nigra (SN) and motor symptoms become more prominent in Parkinson's disease (PD) patients with striatal silent lacunar infarction (SSLI) than in those without SSLI. Hyperhomocysteinemia (HHCY) was an independent risk factor for SSLI in PD patients. In this follow-up study, we investigated the relationship between HHCY and structural changes of the SN in PD patients.
Methods: A total of 72 untreated early PD patients without SSLI, divided into control and HHCY groups, were enrolled in this study. All participants underwent conventional MRI and diffusion kurtosis imaging (DKI) twice; at baseline and at the 2-year visit. The differences of the following variables between the two groups were analyzed: mean kurtosis (MK) values of the SN, the severity of disease, daily dosage of levodopa, and the variation of these indexes from baseline to 2-year visit. Logistic regression analysis was used to identify the relationship between HHCY and structural changes of the SN in PD patients.
Results: 1.All variables mentioned above showed significant differences between the two groups. 2. The variation in MK values of the SN were positively correlated with the variation in the severity of disease. 3. HHCY was an independent risk factor for the variation in MK values of the SN in PD patients.
Conclusion: HHCY is associated with the structural changes of the SN in PD patients. As PD progresses, motor symptoms become aggravated with increased structural changes to the SN, especially in patients with HHCY.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. Although PD is recognized as a neurodegenerative disease, there is increasing evidence that vascular factors play an important role in its development [1]. As early as 2013, animal studies have shown that ischemic lesions of the striatum may affect the substantia nigra (SN) and accelerate the progress of PD [2]. Moreover, our previous study found that striatal silent lacunar infarction (SSLI) was more likely to occur in PD patients with hyperhomocysteinemia (HHCY). As the disease progressed, motor symptoms became more prominent, with increased structural changes to the SN detected by diffusion kurtosis imaging (DKI), especially in patients with SSLI [3].

Recent studies suggested that HHCY may not only be involved in the progression of cerebrovascular diseases by destroying vascular structure [4], but also take part in the neurodegenerative processes by inducing inflammatory reactions and oxidative stress [5,6]. Theoretically, HHCY can promote structural changes of the SN in PD patients by vascular factors and non-vascular neurotoxicity respectively. However, the real effects of HHCY on the progression of the structural changes in the SN in early-stage PD patients remains unknown.

DKI, the further extension of diffusion tensor imaging (DTI), is suitable for the study of both white matter and gray matter [7]. The imaging term “kurtosis” is a dimensionless measure that quantifies the deviation of the water diffusion displacement profile from the Gaussian distribution of unrestricted diffusion, providing a measure of the degree of diffusion hindrance or restriction. Since the deviation from Gaussian behavior is governed by the complexity of the tissue within which the water is diffusing, diffusion kurtosis can be regarded as a measure of a tissue's degree of structure. Mean kurtosis (MK), the average apparent kurtosis along all diffusion gradient encoding directions, is a major parameter of DKI. When structures in the SN become more complex,

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MK values generated from DKI are higher. It is a potential biomarker for imaging studies in the SN of early-stage PD as our recent studies indicated [8]. Furthermore, variation of MK values in the SN, the increased values of MK in the SN during the visit, has been considered as a reliable parameter for quantifying the structural changes of the SN in our 1-year follow-up study [3].

Therefore, in order to reveal the relationship between HHCY and the structural changes of the SN in patients with PD, we conducted a 2-year follow-up study by dynamic detecting the variation of MK values as quantitative indicators of structural changes in the SN [8].

2. Methods

2.1. Patients

The examinations in this study were performed with the understanding and written consent of each participant, with the approval of the First People's Hospital of Foshan Research Ethics Committee. From January 2014 to December 2015, 84 untreated early-stage PD outpatients without SSLI at the Department of Neurology at the First People's Hospital of Foshan were recruited. All outpatients were first diagnosed and had no history of stroke.

The inclusion criteria were as follows: patients were > 50 years of age; they met the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria; and they were Hoehn and Yahr (H—Y) stage 1 or 2. The exclusion criteria were as follows: patients experienced a secondary Parkinson's syndrome or Parkinsonism-plus; they had cardiac insufficiency or severe hepatic and renal impairment; there was a communication barrier; they were unable to complete their MRI; or they had poor compliance.

Demographics (including age, gender, education level, and occupation) were obtained from participants and recorded. Additionally, we evaluated the patients' medical histories for the risk factors of cerebrovascular disease to assess hypertension, diabetes mellitus (DM), hyperlipemia, cigarette smoking, and carotid atherosclerosis.

The onset, disease duration, evolution of the disease, and the daily dosage of levodopa (mg/day) prescribed were elicited in detail and plasma HCY levels were obtained. H—Y staging and the motor section of the Unified Parkinson's Disease Rating Scale (UPDRS III) [9,10] were used for evaluating the severity of motor symptoms in each participant in the OFF condition independently by two investigators who were blinded to the grouping.

2.2. Imaging data acquisition

Participants underwent brain MRI examinations using a 3.0 T superconducting magnetic resonance instrument (GE Signa EXCITE, GE Medical System, USA) with 8-channel phased array coil.

The DKI was obtained using an echo-planar imaging technique with the following scanning parameters: repetition time/echo time, 6500/73.3 ms; motion probing gradients, 25 directions; b values, 0, 1000 and 2000 s/mm^2; field of view, 240 mm^2; matrix size, 1282; slice thickness, 5.0 mm with 0 mm interslice gaps; and one excitation. Image processing operations were performed with an adw 4.5 work station function tool software. In this study, the same Regions of interest (ROIs) selection methods as our previous study was applied, which had been discussed in our paper published in 2015 [8]. ROIs were drawn independently in the bilateral rostral, middle, and caudal SN by two evaluators [11]. The gray map and colour map of MK with marked ROIs in the SN showed an example of typical ROIs measurements on DKI (Fig. 1). Both evaluators agreed upon using this method and were blinded to the grouping.

2.3. Experimental protocol

HHCY was defined as plasma homocysteine (HCY) levels > 15 μmol/L. Participants were divided into two groups at baseline: PD patients with HHCY were assigned into the HHCY group, while those without HHCY were assigned into the control group.

2.4. Follow up

At the 2-year visit, all subjects underwent a second brain conventional MRI examination and DKI using the same magnetic resonance instrument and scanning parameters. Lacunar infarctions with diameter of 3–15 mm in the striatum were counted as SSLI count, according to recently published neuroimaging standards for research into small vessel diseases [12]. Plasma HCY levels, daily dosage of levodopa (mg/day) and MK values in the SN of each subject were obtained again. They were evaluated for motor symptoms in the OFF condition using the H—Y staging and UPDRS III scores again at 2-year visit. Additionally, patients who suffered symptomatic stroke or died from serious disease during this 2-year period, as well as subjects with an incomplete DKI were removed. Ultimately, 38 PD patients with HHCY and 34 PD patients without HHCY were evaluated for this study.

2.5. Outcome measures

The primary outcome was the variation of MK values in the SN. Four secondary outcomes were SSLI, the variation of three indexes: H—Y staging, UPDRS III scores and daily dosage of levodopa. Variation was used to mean the increased values of these variables from baseline to the 2-year visit.

2.6. Statistical analysis

The Statistical Package for the Social Sciences version 21.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Student *t*-test was used to compare the variables at baseline and at the 2-year visit between two groups except gender, which was compared by Chi-square test. Also, the differences in variation of H—Y staging, UPDRS III scores, daily dosage of levodopa and MK values in the SN between two groups were analyzed by Student *t*-test. Spearman correlation analyses between variation of MK values in the SN and variation of H—Y staging or UPDRS III scores were performed. Five risk factors of cerebrovascular disease between two groups were compared by Chi-square test. The relationship between HHCY and the variation of MK values in the SN was determined by multiple logistic regression analysis. The statistic significance was identified with the divergence of $P < 0.05$ (two-tailed test). We also used the mean values of MK in bilateral rostral, middle, and caudal SN as the corrected values for analysis to reduce the errors caused by selection of ROIs.

3. Results

3.1. General characteristics

There were no significant differences in age ($t = 1.371$, $P = 0.175$), gender ($\chi^2 = 0.019$, $P = 0.891$), or disease duration ($t = 0.187$, $P = 0.852$) between two groups. After 2 years, there were 47.4% patients in the HHCY group (18/38) developed SSLI while only 32.4% in the control group (11/34). However, the incidence of SSLI in the HHCY group was not significantly higher at the 2-year visit than that in the control group ($\chi^2 = 1.682$, $P = 0.195$) (Table 1).

3.2. Comparison of the variables between two groups

There were no significant differences in MK values of the SN between two groups at baseline ($t = 0.401$, $P = 0.690$) and at the 2-year visit ($t = 1.004$, $P = 0.319$). H—Y staging for the HHCY group at the 2-year visit was significantly higher than those of the control group ($t = 2.158$, $P = 0.034$) while there were no significant differences initially recorded at baseline ($t = 0.803$, $P = 0.424$). UPDRS III scores for

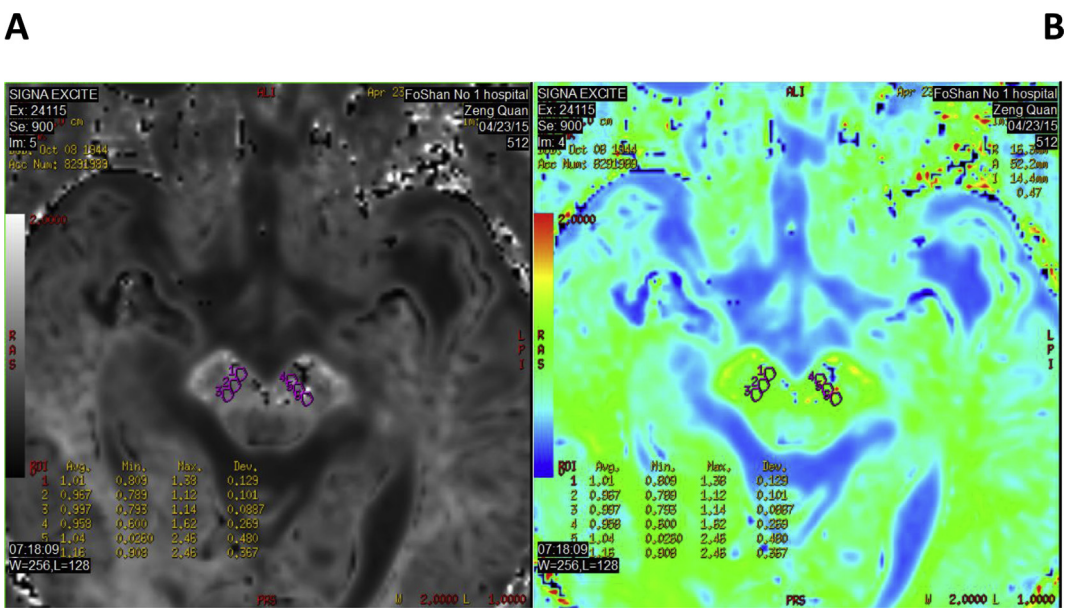

Fig. 1. The gray map(A) and colour map (B) of MK with marked ROI in the SN. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Demographic characteristics of study participants between groups.

Variables	HHCY group	Control group	*P-value*
Gender (M/F)	14/24	12/22	0.891
Age (years)	67.68 ± 5.05	65.97 ± 5.56	0.175
Duration (months)	13.97 ± 3.61	14.12 ± 2.83	0.852
With SSLI after 2 years	47.4% (18/38)	32.4% (11/34)	0.195
Initial plasma HCY levels	17.13 ± 1.24	10.50 ± 1.74	< 0.001
plasma HCY levels after 2 years	20.08 ± 1.34	13.32 ± 1.52	< 0.001
Initial MK values of SN	1.053 ± 0.043	1.057 ± 0.042	*0.690*
MK values of SN after 2 years	1.096 ± 0.047	1.085 ± 0.048	*0.319*
Initial H−Y staging	1.58 ± 0.38	1.65 ± 0.34	0.424
H−Y staging after 2 years	2.08 ± 0.50	1.84 ± 0.44	0.034
Initial UPDRS III scores	14.26 ± 3.05	14.29 ± 2.80	0.964
UPDRS III scores after 2 years	20.11 ± 3.01	18.38 ± 3.29	0.023
daily dosage of levodopa after 2 years	428.95 ± 118.34	355.88 ± 78.59	0.003
Variation in MK values of SN from baseline to 2-year visit	0.044 ± 0.014	0.028 ± 0.014	< 0.001
Variation in H−Y staging from baseline to 2-year visit	0.50 ± 0.29	0.19 ± 0.25	< 0.001
Variation in UPDRS III scores from baseline to 2-year visit	5.89 ± 1.86	4.09 ± 1.55	< 0.001

the HHCY group at the 2-year visit were significantly higher than those of the control group (t = 2.319, $P = 0.023$) while there were no significant differences recorded at baseline (t = 0.045, $P = 0.964$). There were both significant differences in plasma HCY levels between two groups at baseline (t = 18.798, $P < 0.001$) and the 2-year visit (t = 20.052, $P < 0.001$). There were also significant differences in the daily dosage of levodopa (t = 3.048, $P = 0.003$) between two groups at the 2-year visit (Table 1).

3.3. Comparison of the variation between two groups

During the 2-year follow up period, variation in the MK values of the SN ($t = 4.929$, $P < 0.001$), H−Y staging ($t = 4.891$, $P < 0.001$) and UPDRS III scores ($t = 4.457$, $P < 0.001$) of the HHCY group were all significantly higher than those of the control group (Table 1).

3.4. Correlation analyses

Variation in MK values of the SN had a positive correlation with the variation in H−Y staging and UPDRS III scores ($r = 0.350$, $P = 0.003$ and $r = 0.572$, $P < 0.001$, respectively).

3.5. Comparison of risk factors

There were no significant differences in hypertension ($\chi^2 = 0.178$, $P = 0.673$), DM ($\chi^2 = 2.006$, $P = 0.157$), hyperlipemia ($\chi^2 = 0.001$, $P = 0.979$), cigarette smoking ($\chi^2 = 0.243$, $P = 0.622$) and carotid atherosclerosis ($\chi^2 = 0.001$, $P = 0.979$) between two groups (Table 2).

3.6. The relationship between HHCY and the SN

As a result, from baseline to the 2-year visit, MK values of the SN in the HHCY group were more increased by 0.02 than those in the control group. More importantly, HHCY ($P < 0.001$) was determined as an independent risk factor for the variation in MK values of the SN in early-stage PD patients (Table 3).

4. Discussion

In recent years, the role of vascular factors in the development of Alzheimer's disease (AD) has gradually been recognized [13]. As another common neurodegenerative disease, the relationship between PD and vascular factors remains unclear. Some experts believe that vascular factors may not only be associated with vascular Parkinsonism, but also take part in the development of PD [14,15]. The results from animal experiments performed by Beatriz and colleagues strongly supported this idea, as they found that striatal silent infarction and

Table 2
Comparison of five risk factors of cerebrovascular disease between groups.

Variables	HHCY group	Control group	*P-value*
hypertension	22 (57.89%)	18 (52.94%)	0.673
DM	22 (57.89%)	14 (41.18%)	0.157
hyperlipemia	20 (52.63%)	18 (52.94%)	0.979
cigarette smoking	6 (15.79%)	4 (11.76%)	0.622
carotid atherosclerosis	20 (52.63%)	18 (52.94%)	0.979

Table 3
Analyze the relationship between HHCY and MK values of the SN using multiple logistic regression.

	Non-adjusted	Adjust I	Adjust II
B(95% CI)P value	0.02 (0.01,0.02) < 0.001	0.02 (0.01,0.02) < 0.001	0.02 (0.01,0.02) < 0.001

Non-adjusted model adjust for: None.
Adjust I model adjust for: Gender, Age, Hypertension, DM, Hyperlipemia, Smoking.
Adjust II model adjust for: Gender, Age, Hypertension, DM, Hyperlipemia, Smoking, Carotid Atherosclerosis.

dopamine neuron degeneration of the SN might be closely related [2]. Our previous study also found that early PD patients with SSLI had more prominent structural changes in the SN and more aggravated motor symptoms [16], providing a clinical basis for the involvement of vascular factors in PD.

In order to study the effects of vascular factors in the progression of PD, our 1-year follow-up study indicated that HHCY was an independent risk factor for SSLI in patients with early PD [3]. It is well known that HCY is a sulfur-containing amino acid formed by the interconversion of methionine and cysteine. Normally the human plasma HCY concentration generally does not exceed 15 μmol/L [17]. Pathophysiologically, elevated HCY damages the vascular structure by destroying vascular endothelial cells. Furthermore, inflammatory reactions and oxidative stress induced by HHCY can cause non-vascular neurotoxicity [18,19]. As a result, HHCY is not only an independent risk factor for cerebrovascular diseases [4], but may also promote the progression of neurodegenerative diseases, including aggravating PD through apoptosis [17] and excitotoxic amino acid toxicity [19,20]. It is noteworthy that the epidemiological studies show that there is a high incidence of HHCY in China [21] because: 1) the Chinese diet has a low intake of folic acid diet; 2) the gene encoding HCY metabolism-related enzyme (MTHFR C677T) has a high mutation rate in the Chinese population [22]. Thus, PD patients in China are more likely to develop HHCY. In theory, HHCY may either induce secondary injury to the SN by developing SSLI or directly contribute to the progress of degeneration in the SN in PD patients. Therefore, to clarify the relationship between HHCY and structural changes in the SN detected by MK values of DKI are of crucial importance for better preventing progression of PD.

Given that the relationship between HHCY and the SN might be influenced by SSLI, we recruited PD patients without SSLI as study subjects to reduce the interference of SSLI. In addition, in PD patients undergoing levodopa therapy, plasma HCY are elevated as a result of the transmethylation of levodopa via catechol O-methyl transferase. In order to avoid the influence on HCY by levodopa [23,24], in this study untreated PD patients were recruited and divided into two groups according to their plasma HCY obtained at baseline. Even though plasma HCY of each participant was tracked at 2-year visit, we didn't focus on the difference between two groups for considering the influence by levodopa.

Baseline data from this study indicated that 52.8% of the participants who completed the follow-up were complicated with HHCY, reflecting the high prevalence of HHCY in PD patients in the region. Meanwhile, we found that basic characteristics, such as gender, age, course of disease, and baseline MK values of the SN, HY staging, UPDRS III scores of two groups were consistent. However, follow-up data showed that compared with the control group, H—Y staging, UPDRS III scores and the daily dosage of levodopa in HHCY group were significantly increased after 2 years. Although it was found that the incidence of SSLI in the HHCY group was not significantly higher than that in the control group at the 2-year visit, PD patients with HHCY had an increased risk of SSLI compared with those without HHCY during this period (47.4% vs 32.4%). This increasing trend suggested that positive statistical results might need a longer follow-up period.

It was also found in this study that in most of participants, the original MK values of the SN were around 1.0 while the variation of MK values in the SN ranged just between 0.02 and 0.05. It meant that 2 years later, MK values of the SN only increased by 2–5% of the original MK values of the SN at baseline. So when we compared the difference of the original MK values in the SN between two groups after 2 years (1.096 ± 0.047 vs 1.085 ± 0.048), there were no significant differences. However in a 1-year follow-up study, it was found that variation of MK values in the SN was a sensitive variable for detecting the structural changes of the SN [3], which positively correlated with the severity of motor symptoms. Compared with the original MK values of the SN, the variation of MK values in the SN, which was the primary outcome, should be much more important in this study because it quantitatively reflected the severity of structural changes in the SN during 2 years. As a result, the variation of MK values in the SN of the HHCY group were significantly higher than those of the control group (0.044 ± 0.014 vs 0.028 ± 0.014), which meant the structural changes of the SN in the HHCY group were much more apparent than those in the control group.

Because the most significant pathological changes in PD patients occur in the SN, which is associated with motor symptoms, the relationship between the variation of MK values in the SN and the severity of motor symptoms (which are usually evaluated by H—Y staging and UPDRS III scores) should be analyzed in this study. Compared with patients without HHCY, patients with HHCY showed greater 2-year increase in these three indexes. Furthermore, correlation analysis demonstrated that the variation of MK values in the SN were positively correlated with the changes in both HY staging and UPDRS III scores, suggesting that as PD progressed, structural changes in the SN were related to the progress of motor symptoms, regardless of the combination of HHCY. However, these phenomena were much more apparent in PD patients with HHCY than in the control group patients. Besides the severity of motor symptoms, daily dosage of levodopa was considered as another indicator for the severity of PD. In result, as the progression of disease, daily dosage of levodopa in PD patients with HHCY increased more than those in PD patients without HHCY.

Considering the results of five common vascular risk factors analysis, the two groups showed basically the same risks in hypertension, diabetes, hyperlipidemia, smoking, and carotid atherosclerosis between at baseline. Most importantly, it was found that HHCY was an independent risk factor for increased values of MK in the SN in PD patients with early-stage during the 2-year period by adjusting factors such as gender, age and five common vascular risk factors, which implied that HHCY was associated with the structural changes of the SN in PD patients.

Based on the results of our 1-year follow-up study, we put forward the hypothesis that HHCY might induce secondary injury to the SN by developing SSLI and then accelerate progression of PD. But this study may add to our previous hypothesis. In this study we found that even though there were no difference in SSLI between two groups 2 years later, the structural changes of the SN in the HHCY group were much more apparent than those in the control group. The interpretation for these new findings might be as follows: HHCY is likely to be involved in the course of structural changes to the SN and accelerate the progression of PD, not only by inducing the develop of SSLI but also by directly promoting the degeneration of the SN. However, the effect of HHCY on the variation of MK values in the SN and the possibility of this new hypothesis should be tested in the future study.

Although there is insufficient evidence from previous studies showing that vascular factors can play a direct role in the development of PD [25,26], our previous study [3] and recent follow-up suggest that silent cerebral ischemic lesions such as SSLI and vascular risk factors such as HHCY are likely to affect the structure and function of the SN in PD patients. Of course, there are still limitations in this study. In the future, we should carry out multicenter studies, increase the number of

cases, extend the follow-up time, and improve the research methods to provide a more convincing basis for further clarifying the relationship between vascular factors and the evolution of PD.

5. Conclusions

In PD patients, HHCY is associated with the structural changes to the SN, detectable by DKI. With the progression of PD, motor symptoms and daily dosage of medication become more serious with increased structural changes to the SN. However, this phenomenon is more prominent in early-stage PD patients with HHCY.

Authors' roles

1) Research project: A. Conception (Guohua Zhang), B. Organization (Chengguo Zhang, Yukai Wang), C. Execution (Lijuan Wang, Yuhu Zhang);

2) Statistical Analysis: A. Design (Haiqun Xie), B. Execution (Jiancong Lu), C. Review and Critique (Kun Nie);

3) Manuscript: A. Writing of the first draft (Guohua Zhang), B. Review and Critique (Yukai Wang).

Conflicts of interest/financial disclosures

The authors declare that there is no conflict of interest and financial disclosures regarding the publication of this article.

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