RESEARCH ARTICLE

Association of Homocysteine With Ventricular Dilatation and Brain Atrophy in Parkinson's Disease

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ABSTRACT: Parkinson's disease (PD) patients are treated with levodopa (L-dopa) to help stabilize their impaired motor abilities; however, L-dopa leads to increased homocysteine (Hcy) levels, which may have a deleterious effect on brain structure and function. The purpose of this study was to examine the impact of increased Hcy concentration on global brain atrophy as determined by magnetic resonance imaging in PD patients and controls. The effect of high Hcy level on ventricular dilatation (percentage of intracranial volume [%ICV]) and total tissue volume (%ICV) was examined at baseline and longitudinally at 36 months. Age, sex, education, and L-dopa duration (in PD patients) were included as covariates. Elevated Hcy levels correlated positively with ventricular dilatation (%ICV) in the whole sample ($P = 0.004$) and in the PD group ($P = 0.008$). At baseline, adults with a high Hcy level (>14 μmol/L) had higher ventricular volume (%ICV) than adults with a low Hcy level (≤14 μmol/L) in the whole sample ($P = 0.006$) and in the PD group ($P = 0.03$), which persisted over 36 months in both the whole sample ($P = 0.004$) and the PD group ($P = 0.03$). PD patients with high Hcy concentrations had a greater rate of ventricular enlargement (%ICV) over time compared with those with low Hcy concentration ($P = 0.02$). Elevated Hcy concentration was associated with increased ventricular dilatation (%ICV) in PD patients. A larger sample with a broader age range and longer follow-up is needed to establish the consequences of high Hcy level, including interactions with genetic and environmental risk factors, in PD. © 2014 International Parkinson and Movement Disorder Society

Key Words: Parkinson's disease; homocysteine; magnetic resonance imaging; brain atrophy; longitudinal study

The impact of genetic and environmental influences on neurodegenerative diseases in older adults is at the forefront of growing research.[1,2] Parkinson's disease (PD) is a neurodegenerative condition associated with loss of dopamine-producing cells within the substantia nigra leading to motor impairment.[3-5] PD patients are usually treated with the precursor levodopa (L-dopa) to replace the loss of dopamine.[6] L-Dopa is metabolized via decarboxylation and methylation, in which the catechol o-methyltransferase enzyme catalyzes the methylation of L-dopa.[7,8] In turn, involvement of this metabolic pathway increases the production of homocysteine (Hcy),[7,8] an excitatory amino acid that activates the *N*-methyl-D-aspartate receptor, which may be associated with neurotoxicity and increased risk of cerebrovascular events.[9] Thus, PD patients have elevated Hcy levels because of L-dopa treatment.[10] Additional factors affecting Hcy concentration include genetic polymorphisms, coding for methylenetetrahydrofolate reductase[11] and reduced intake of B vitamins.[12,13] High Hcy levels have also been linked to cognitive impairment[14] and hippocampal atrophy in older adults without PD.[15] Nondemented

Additional Supporting Information may be found in the online version of this article.

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older adults with Hcy levels greater than 14 μmol/L performed poorly on measures of psychomotor speed and memory.[14]

Elevated Hcy concentration is a risk factor for Alzheimer's disease (AD), dementia,[16-18] and global brain atrophy as displayed with magnetic resonance imaging.[19] Recently, Rajagopalan and colleagues examined the relationship between Hcy level and brain atrophy in 732 older adults with AD, older adults with mild cognitive impairment (MCI), and healthy controls from the Alzheimer's Diseases Neuroimaging Initiative. Hcy level was greater in AD and MCI patients than in healthy controls and was associated with more frontal, parietal, and occipital white matter atrophy.[16] Older MCI adults with elevated Hcy level (>14 μmol/L) showed the highest amount of overall brain atrophy.[16] Furthermore, a recent randomized and double-blind controlled study showed decreased Hcy levels and slowed brain atrophy in older adults with MCI with B-vitamin supplementation.[20]

The increase in Hcy levels because of L-dopa treatment with or without additional environmental risk factors such as insufficient vitamin B12 or folate places PD patients at high risk for brain atrophy, which is accelerated in older adults[21] and in PD dementia.[22] The effect of elevated Hcy concentrations on brain atrophy has not been studied in PD.[17] The goal of the present study was to examine the association between Hcy level and brain atrophy in PD patients and controls. Whole-brain atrophy and ventricular dilatation for high Hcy level was examined in PD patients and healthy controls at baseline and longitudinally. We studied the relationship between Hcy concentration and overall atrophy in PD and control groups to examine the hypothesis that higher Hcy levels are associated with a greater degree of atrophy cross-sectionally. Next, we tested whether elevated Hcy concentration was associated with decreased tissue volume or enlarged ventricles over time, independent of other risk factors for atrophy.

Patients and Methods

Participants

PD patients (n = 50) and controls (n = 50) aged 64 to 84 years old (mean age, 71.3 ± 4.5 years; 43% female) volunteered for a 3-year longitudinal study in Edmonton, Alberta, Canada, from April 2003 to September 2009.[23] PD patients were recruited from movement disorder clinics, the Parkinson's Society of Alberta, and community neurologists. The control subjects were recruited from contacts of the PD patients and through advertisements in general medical clinics and senior centers. Controls did not have cognitive complaints or exclusionary medical conditions and were age- and sex matched to PD patients.[12] This study was in agreement with the University of Alberta's Health Ethics review board. Signed informed consent was required from all participants prior to the study. Four participants determined to have atypical parkinsonism and 1 control with unusually large ventricles were excluded, leaving a total of 95 participants (PD patients, n = 46; controls, n = 49) for the cross-sectional study. Eleven participants (PD patients, n = 7; controls, n = 4) did not complete the longitudinal part of the study, leaving 84 participants for the longitudinal analyses. One participant was too frail to attend the follow-up scan, 2 dropped out, 2 received cardiac pacemakers, and 6 died before the longitudinal follow-up. They did not differ in age, sex, or cognitive status (data not shown). Details regarding participants and recruitment procedures have been published.[12,23]

Magnetic Resonance Imaging Measures

A Siemens Sonata 1.5-T system was used to obtain all the images at baseline, 18 months, and 36 months. Only images from baseline and 36 months were examined in the present study. Measurements of (1) whole-brain tissue volume, (2) ventricular volume, and (3) whole-brain tissue volume and ventricular volume as a percentage of intracranial volume (ICV) were examined for cross-sectional and longitudinal analyses. White-matter change at baseline was rated using the Wahlund age-related white matter rating scale as previously described.[24,25] Details regarding MRI measurements have been published.[23,25] In brief:

1. Whole-brain gray- and white-matter volumes were determined from the T1-weighted images using the automated segmentation feature in the program SPM5 (Wellcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm/).
2. Lateral and third ventricles were manually outlined on the T1-weighted images using DISPLAY software (available at http://www.bic.mni.mcgill.ca/ServicesSoftware/HomePage).
3. Intracranial volumes were measured from T1-weighted images using DISPLAY software.

Laboratory Measurements

Blood was obtained at baseline to determine Hcy, folate, creatine, and vitamin B12 levels after an overnight fast from food but not medication. Hcy, folate, and vitamin B12 levels were measured from blood plasma, which was immediately stored on ice and centrifuged within an hour of collection.[12] DNA was extracted from all participants to determine allelic differences (ε2, ε3, ε4) for the apolipoprotein (*APOE*) gene.[12]

TABLE 1. Descriptives and clinical characteristics at baseline and 36 months (mean ± standard deviation) for the whole sample, PD patients and control subjects

	Baseline				36 Months			
Measure	Whole sample	PD	Controls	*P*	Whole sample	PD	Controls	*P*
Number	95	46	49	—	84	38	44	—
Male/female	54/41	26/20	28/21	1.0	48/36	22/17	26/19	0.7
Age (y)	71.31 ± 4.54	70.97 ± 4.26	71.63 ± 4.80	0.4	73.91 ± 4.24	73.68 ± 3.82	74.35 ± 4.52	0.5
Education (y)	14.63 ± 3.26	14.13 ± 2.96	15.10 ± 3.48	0.1	14.63 ± 3.26	14.13 ± 2.96	15.10 ± 3.48	0.1
Hcy (μmol/L)	12.13 ± 3.67	13.92 ± 3.81	10.45 ± 2.57	0.0	—	—	—	—
APOE ε4 (none/1 allele/2 alleles)	75/17/2	40/5/1	35/12/1	0.09	66/15/2	34/4/1	32/11/1	0.2
PD duration (y)	N/A	8.42 ± 4.51	N/A	N/A	N/A	10.37 ± 5.50	N/A	N/A
UPDRS part 3	9.15 ± 9.42	16.61 ± 8.09	2.14 ± 2.99	0.0	10.37 ± 11.41	19.45 ± 10.70	2.52 ± 3.30	0.0
L-dopa equivalents (mg)	N/A	627.45 ± 333.31	N/A	N/A	N/A	870.49 ± 525.64	N/A	N/A
L-dopa total dose (mg)	N/A	526.79 ± 302.15	N/A	N/A	N/A	773.08 ± 526.69	N/A	N/A
L-dopa duration (y)	N/A	4.78 ± 4.18	N/A	N/A	N/A	7.00 ± 4.68	N/A	N/A
Folate (nmol/L)	862.80 ± 221.25	833.24 ± 209.92	890.55 ± 230.06	0.2	—	—	—	—
Vitamin B12 (pmol/L)	341.43 ± 165.10	297.35 ± 123.29	382.82 ± 188.47	0.01	—	—	—	—
MMSE	28.36 ± 1.64	28.17 ± 1.75	28.54 ± 1.53	0.3	27.31 ± 2.71	26.76 ± 3.46	27.75 ± 1.83	0.1
Intracranial volume (ICV), cm^3	1518.80 ± 149.38	1522.66 ± 149.05	1515.17 ± 151.15	0.8	N/A	N/A	N/A	N/A
Total ventricle volume, mL	37.90 ± 15.76	38.72 ± 15.68	37.13 ± 15.96	0.6	42.04 ± 18.36	43.28 ± 18.79	40.97 ± 18.13	0.6
Total tissue volume, L	1.08 ± 0.11	1.08 ± 0.11	1.09 ± 0.11	1.0	1.05 ± 0.11	1.05 ± 0.10	1.05 ± 0.11	1.0
Total ventricle volume, %ICV	2.48 ± 0.94	2.54 ± 0.98	2.41 ± 0.90	0.5	2.75 ± 1.10	2.84 ± 1.18	2.66 ± 1.03	0.5
Total tissue volume % ICV	71.49 ± 3.48	71.30 ± 3.12	71.66 ± 3.80	0.6	69.56 ± 3.64	69.63 ± 3.20	69.51 ± 4.03	0.9
White matter rating	3.60 ± 3.47	3.76 ± 3.88	3.45 ± 3.08	0.7	—	—	—	—

PD, adults with Parkinson's disease; Hcy, homocysteine; *APOE* ε4, apolipoprotein ε4; L-dopa, levodopa; MMSE, Mini Mental State Exam; ICV, intracranial volume; UPDRS, Unified Parkinson's Disease Rating Scale.

Statistical Analysis

Baseline demographics were computed for the whole sample (n = 95) and 2 subgroups—PD patients (n = 46) and control subjects (n = 49) (see Table 1); and subjects with high (>14 μmol/L) versus low (≤14 μmol/L) Hcy level[12,14,26] (Table 2). SPSS 16.0 for Windows (SPSS Inc., Chicago, IL) was used for all analyses, and statistical significance was tested at $P < 0.05$. To account for confounding variables, partial correlations, cross-sectional analysis of covariance (ANCOVA), and longitudinal repeated-measures analysis of covariance (rANCOVA) were covaried with age, sex, education, and L-dopa duration (for comparisons within the PD group only). To examine the robustness of our findings, as secondary analyses we included the Mini-Mental State Examination (MMSE), Unified Parkinson's Disease Rating Scale part 3, and white matter ratings as additional covariates with age, sex, and education. Furthermore, we also examined the same set of secondary analyses with PD disease duration (for comparisons within the PD group only) instead of L-dopa duration to determine whether the use of L-dopa duration versus PD disease duration changed the findings.

Cross-Sectional

First, partial correlation coefficients between Hcy and ventricle volume (%ICV) and whole-tissue volume (%ICV) were computed for the whole sample, followed by the 2 subgroups. Second, 2-factor ANCOVA was evaluated with 2 levels of Hcy (high versus low) entered as independent factors and ventricular volume or total tissue volume as dependent factors. Hcy main effect was examined on total ventricular volume (%ICV) and total tissue volume (%ICV).

Longitudinal

Two rANCOVA were computed to examine longitudinal changes. First, we examined the change in total ventricular volume (%ICV), followed by change in total tissue volume (%ICV), over 36 months in the whole sample and the 2 subgroups. Hcy level at baseline was specified as a between-subject factor with 2 levels (high versus low), and the dependent variable was either ventricular volume or total tissue volume as a within-subject factor with 2 levels (measured at baseline and 36 months). Second, the interaction between Hcy level and brain atrophy change was examined to assess the

TABLE 2. Baseline descriptive and clinical characteristics (mean ± standard deviation) for the whole sample, PD patients, and control subjects by high (>14 μmol/L) and low Hcy (≤14 μmol/L)

	Whole sample			PD			Controls		
Measure	Low Hcy	High Hcy	*P*	Low Hcy	High Hcy	*P*	Low Hcy	High Hcy	*P*
Number	69	26	—	25	21	—	44	5	—
Male/female	37/32	17/9	0.3	12/13	14/7	0.2	25/19	3/2	0.9
Age (y)	70.66 ± 4.29	73.04 ± 4.82	0.02	70.21 ± 4.49	71.88 ± 3.89	0.2	70.91 ± 4.20	77.92 ± 5.73	0.001
Education (y)	14.94 ± 3.42	13.81 ± 2.68	0.1	14.88 ± 3.27	13.24 ± 2.32	0.06	14.98 ± 3.54	16.20 ± 3.03	0.5
Hcy (μmol/L)	1 0.35 ± 2.20	16.86 ± 2.30	0.0	11.07 ± 2.04	17.33 ± 2.31	0.0	9.945 ± 2.20	14.88 ± 0.61	0.0
APOE ε4 (none/1 allele/2 alleles)	53/13/2	22/4/0	0.5	22/2/1	18/3/0	0.8	31/11/1	4/1/0	0.7
PD duration (y)	N/A	N/A	N/A	7.64 ± 4.46	9.34 ± 4.50	0.2	N/A	N/A	N/A
L-dopa equivalents (mg)	N/A	N/A	N/A	538.24 ± 284.16	746.40 ± 363.97	0.04	N/A	N/A	N/A
L-dopa total dose (mg)	N/A	N/A	N/A	437.50 ± 266.52	645.83 ± 312.75	0.03	N/A	N/A	N/A
L-dopa duration (y)	N/A	N/A	N/A	3.74 ± 3.94	6.01 ± 4.22	0.066	N/A	N/A	N/A
Folate (nmol/L)	881.90 ± 191.32	812.12 ± 284.29	0.2	878.92 ± 183.25	778 ± 230.52	0.1	883.59 ± 197.81	951.80 ± 457.42	0.5
Vitamin B12 (pmol/L)	359.13 ± 150.25	294.46 ± 194.76	0.09	317.56 ± 112.90	273.29 ± 133.37	0.2	382.75 ± 164.35	383.40 ± 368.63	1.0
MMSE	28.52 ± 1.48	27.91 ± 2.00	0.1	28.50 ± 1.64	27.72 ± 1.84	0.2	28.54 ± 1.40	28.60 ± 2.61	0.9
Intracranial volume (ICV) (cm^3)	1507.06 ± 149.17	1549.93 ± 148.29	0.2	1493.01 ± 142.74	1557.96 ± 152.09	0.1	1515.04 ± 153.74	1516.23 ± 141.52	1.0
White matter rating	3.61 ± 3.68	3.58 ± 2.93	1.0	4.56 ± 4.61	2.81 ± 2.56	0.1	3.07 ± 2.94	6.80 ± 2.17	0.009

PD, adults with Parkinson's disease; Hcy, homocysteine; *APOE* ε4, apolipoprotein ε4; L-dopa, levodopa; MMSE, Mini Mental State Exam; ICV, intracranial volume.

rate of atrophy in high versus low Hcy groups in the whole sample and the 2 subgroups.

Results

Study Population

Subject characteristics are presented in Table 1. The PD and control populations were similar in age, sex distribution, education, and follow-up (Table 1). The 16 excluded participants, 4 from baseline analyses, and 11 in the longitudinal analyses, were older (mean, 74.60 ± 6.01 years; $P = 0.003$), and had lower MMSE scores (mean, 27.12 ± 1.89; $P = 0.03$), but did not differ in proportions of low and high Hcy levels (6 high/10 low; $P = 0.3$), sex distribution (10 men/6 women; $P = 0.7$), or education (mean, 13.63 ± 2.68 years; $P = 0.2$). Ten PD subjects and 3 controls with complete follow-up had developed significant cognitive impairment or dementia as previously reported.[23] We had 2 levels (baseline and 36 months) for both the tissue volume (%ICV), and ventricular volume (%ICV) repeated-measures factors. Sphericity assumptions were met (i.e., differences in variance between the 2 levels were equal).

Cross-Sectional

Partial Correlations

Increasing Hcy levels were positively correlated with total ventricle volume (%ICV) for the whole sample ($r_{90} = 0.3$, $P = 0.004$) and the PD subgroup ($r_{40} = 0.40$, $P = 0.008$), but not with the control group ($r_{44} = 0.16$, $P = 0.3$) (see Figure 1). No significant correlations were observed for total tissue volume (%ICV).

Baseline

Participants with elevated Hcy concentration had higher total ventricle volume (%ICV) (mean, 2.92% ± 0.87%) than those with low Hcy concentration (mean, 2.31% ± 0.92%) in the whole sample ($F_{1,90} = 7.83$, $P = 0.006$, $\eta_p^2 = 0.08$). The main effect of Hcy was present in the PD subgroup: higher ventricle volume (mean, 2.84% ± 0.93%) was associated with elevated Hcy level and lower ventricle volume (mean, 2.28% ± 0.97%) with low Hcy level ($F_{1,40} = 4.91$, $P = 0.03$, $\eta_p^2 = 0.11$). The Hcy effect on ventricular volume was not present in the control subgroup ($F_{1,42} = 2.52$, $P = 0.1$, $\eta_p^2 = 0.06$), nor was it significantly associated with total tissue volume in the whole sample or the 2 subgroups (Table 3).

Longitudinal

The main effect of Hcy was significant in the whole group ($F_{1,79} = 8.99$, $P = 0.004$, $\eta_p^2 = 0.10$) and in the PD group ($F_{1,33} = 5.44$, $P = 0.03$, $\eta_p^2 = 0.14$); see Figure 2 and Table 3. Ventricular volume (%ICV) did not increase significantly over 36 months for the high and low Hcy groups in the whole sample ($F_{1,79} = 0.26$, $P = 0.61$, $\eta_p^2 = 0.003$), the PD ($F_{1,33} = 0.009$, $P = 0.9$, $\eta_p^2 = 0.00$), or the control ($F_{1,38} = 0.95$, $P = 0.3$, $\eta_p^2 = 0.02$) subgroups. However, in the PD subgroup, the interaction between Hcy level and ventricle volume change (%ICV) showed that the high Hcy-level group had a higher rate of ventricular dilatation over the 36-month period than the low Hcy-level group ($F_{1,33} = 5.76$, $P = 0.02$, $\eta_p^2 = 0.15$). An average increase of 0.40 (%ICV) was

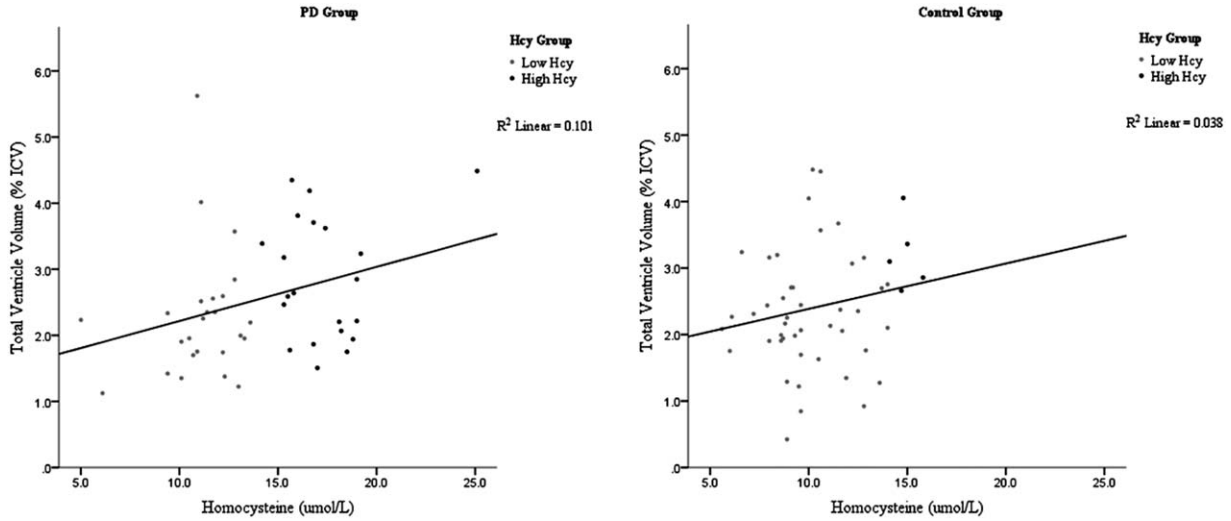

FIG. 1. A significant positive linear correlation between Hcy concentration (low to high) and total ventricle volume (% ICV) was observed in the PD group at baseline.

observed for the high Hcy-level group and 0.22 (%ICV) for the low Hcy-level group over 36 months. There was no significant interaction between Hcy level and ventricle volume change in the whole sample ($F_{1,79} = 3.44$, $P = 0.07$, $\eta_p^2 = 0.04$) or in the control group ($F_{1,38} = 0.015$, $P = 0.9$, $\eta_p^2 = 0.00$). No significant differences were observed for low versus high Hcy level on total tissue volume (%ICV) change in the whole sample or in the 2 subgroups (see Table 3).

Secondary Analyses

Secondary analyses confirmed our initial findings. All cross-sectional and longitudinal results were not affected by adding MMSE, Unified Parkinson's Disease Rating Scale part 3, and white matter rating at baseline as covariates or replacing L-dopa duration with PD disease duration as a covariate in the PD subgroup (see supplementary tables).

Discussion

The purpose of this study was to examine the effect of Hcy level on total tissue volume (%ICV) and ventricular dilatation (%ICV) in PD patients and healthy controls. Ventricular volume (%ICV) was greater in adults with high Hcy levels. This association was present in PD patients, who had higher Hcy levels, but not in the control subgroup. Although we examined Hcy level, which has been shown to increase with L-dopa treatment,[23] we acknowledge that this increase may also have been a result of reduced Hcy metabolism potentially related to lack of dietary vitamins, genetic

TABLE 3. Summary of cross-sectional and longitudinal results for Hcy and ventricle volume (%ICV) in the whole sample and 2 subgroups (PD and controls)

	Whole sample		PD		Controls	
	Low Hcy	High Hcy	Low Hcy	High Hcy	Low Hcy	High Hcy
Cross-sectional analysis						
Number of subjects	69	26	25	21	44	5
Total ventricle volume (%ICV)	2.31 ± 0.92	2.92 ± 0.87	2.28 ± 0.97	2.84 ± 0.93	2.28 ± 0.85	3.21 ± 0.54
P	0.006		0.03		0.1	
Longitudinal analysis						
Number of subjects	64	20	23	16	39	4
Total ventricle volume (%ICV), baseline	2.28 ± 0.91	3.01 ± 0.88	2.27 ± 1.01	2.94 ± 0.94	2.24 ± 0.81	3.29 ± 0.58
Total ventricle volume (%ICV), 36 months	2.53 ± 1.04	3.39 ± 0.99	2.49 ± 1.11	3.34 ± 1.07	2.50 ± 0.96	3.62 ± 0.59
P of main effect of Hcy group	0.004		0.03		0.09	
P of ventricle volume (%ICV) change from baseline to 36 months	0.61		0.93		0.34	
P of interaction of Hcy group with scan time points (at baseline and 36 months)	0.07		0.02		0.90	

PD, adults with Parkinson's disease; Hcy, homocysteine; ICV, intracranial volume.
All results shown are covaried for age, sex, education, and L-dopa duration (in the PD group).

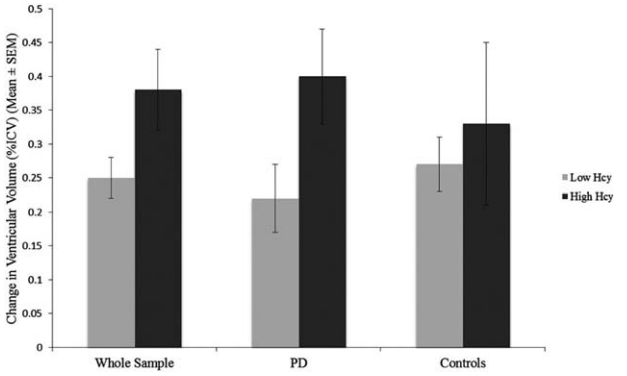

FIG. 2. Change in ventricle volume (%ICV) at 36 months for the whole sample and the 2 subgroups: PD patients and controls. The error bars represent the standard error of the mean (SEM).

risk factors, or factors related to treatment or disease duration, including cognitive impairment. Camicioli, Bouchard, and Somerville examined the same group of participants and found that adults taking B vitamins had lower Hcy levels in both the PD and control groups.[12] Although the modulating effect of B vitamins may have reduced the effect of Hcy level in our study population, this provided a broad range of Hcy concentrations.

In the PD group, ventricular dilation in the group with higher Hcy levels was accelerated compared with the group with lower Hcy levels, independent of age, sex, education, and L-dopa treatment duration. Thus, high Hcy levels in PD patients may increase their risk for ventricular dilatation.[23] Our results are consistent with findings associating high Hcy level with more brain atrophy.[15,16] It remains possible that although L-dopa influences Hcy levels, the relationship to ventricular size might be coincidental (ie, related to increased disease severity, which would be associated with increased atrophy and increased L-dopa doses and hence increased Hcy levels).

The loss of total tissue volume was not associated with high Hcy level, in contrast to our findings for ventricular dilatation. One explanation for this unexpected result could be the lower power to detect the comparatively small amount of total tissue loss relative to the overall amount of brain tissue volume. Ventricular volumes are relatively smaller compared with tissue loss; therefore, a statistically significant difference in ventricular dilatation was observed. A recent autopsy longitudinal study with 71 healthy older adults observed a robust correlation between ventricular volume trajectory and pathological measures of AD and vascular disease. However, the same type of association was not observed for total brain volume and hippocampal volume, suggesting that ventricular volume alone is a more sensitive measure.[27] Future studies should explore ventricular volume as a more vulnerable and possible early marker for neurodegeneration in different clinical and healthy older populations.

A previous study showed that PD patients with MCI have enlarged ventricles.[28] It is likely that a proportion of our subjects had MCI, although only a few had a clinical dementia rating scale score of 0.5 at baseline. We chose not to further subdivide our subjects at baseline into those with and without MCI given our small sample size with incident significant cognitive impairment. Our previous cross-sectional study in this sample showed that Hcy levels were not related to cognitive impairment.[12] In addition, a recent study observed no association between disease duration and white-matter hyperintensities.[29] Similarly, white-matter rating did not affect our findings. In our primary analysis, we chose to include L-dopa duration (similar to our main analyses) as a covariate instead of PD duration, because this would be expected to be associated with elevated Hcy level, but secondary analyses using disease duration did not alter our results.

Hcy levels may interact with other genetic risk factors causing brain atrophy. Wilhelm and colleagues studied gene–environment interactions by examining the *APOE* gene in combination with Hcy levels among 52 patients with chronic alcoholism. Patients with elevated Hcy levels in the presence of the *APOE* ϵ4 genotype had smaller hippocampal volumes.[30] The ϵ4 allele of the *APOE* gene is a risk factor for the development of sporadic AD, which is associated with an increased rate of brain atrophy.[31] In addition to a higher proportion with an *APOE* ϵ4 genotype (60% in AD patients versus 10% in controls), Hcy levels may also be elevated in individuals with AD.[32] Weiner and colleagues observed a significant main effect for Hcy concentration and an interaction between Hcy and *APOE* ϵ4 on total hippocampal loss in AD patients.[30] Only few individuals in our PD sample had 1 or more copies of the *APOE* ϵ4 allele, consistent with other studies of PD, in which the proportion of patients with an *APOE* ϵ4 allele was not elevated[33]; therefore, we did not have sufficient power to examine the effect of *APOE* ϵ4 alone or in interaction with Hcy level. We acknowledge that the presence of the *APOE* ϵ4 risk allele could potentially have influenced the results. Future research should examine the effect of *APOE* ϵ4 risk allele in interaction with Hcy level on PD patients over time.

The present study included participants from an older cohort (eg, 64 to 84 years old), which may have affected the correlations observed with brain atrophy; however, our findings in the whole sample and the PD group were not affected by covarying for age, sex, education, and L-dopa duration (PD group only). In addition, we did not observe a signficant difference in ventricular volume in the control group, which may be partly a result of insufficient power, as only 5 subjects were in the high Hcy group. A weakness of our study was that Hcy level was only measured at baseline; however, an increase in the rate of ventricular

volume was still observed for PD patients in the the high Hcy group. Ideally, future studies should examine Hcy level as a continous measure, as it is more robust and uses the full range of subject data. Other metabolites associated with L-dopa exposure, such as methylmalonic acid, may be better correlated with neuropathy present in idiopathic PD[34] but have not been examined with regard to brain atrophy.

Although the effect of elevated Hcy level on brain atrophy has been previously examined, the present study is the first to examine Hcy effects on MRI measures longitudinally in a group of PD patients. Our results are consistent with an effect of Hcy on ventricular volume, a correlate of cognitive decline in PD. Future research should examine a larger sample size of both PD patients and controls longitudinally to better delineate the moderating roles of sex, age, genetic polymorphisms, cognitive impairment, and motor subtypes. Examining incident cases would allow the examinaton of changes assocated with the introducton of L-dopa.

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