

Methylation Status and Neurodegenerative Markers in Parkinson Disease

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BACKGROUND: Increased concentrations of plasma total homocysteine (tHcy) have been associated with age-related diseases, including dementia, stroke, and Parkinson disease (PD). Methylation status might link Hcy metabolism to neurodegenerative proteins in patients with PD.

METHODS: We tested blood samples from 87 patients with PD (median age 68 years; 35 men) for tHcy, methylmalonic acid (MMA), vitamin B₁₂, vitamin B₆, folate, S-adenosyl methionine (SAM), S-adenosyl homocysteine (SAH), and amyloid- β (1–42). We collected citrate blood from a subset of 45 patients to prepare platelet-rich plasma, and we used washed platelets to prepare cell extracts for amyloid precursor protein (APP) and α -synuclein assays. We used brain parenchyma sonography to estimate the substantia nigra echogenic area in a subset of 59 patients.

RESULTS: Serum concentrations of tHcy were increased in PD patients (median 14.8 μ mol/L). tHcy (β coefficient = -0.276) and serum creatinine (β = -0.422) were significant predictors of the ratio of SAM/SAH in plasma ($P < 0.01$). The plasma SAM/SAH ratio was a significant determinant for DemTect scores (β = 0.612 , $P = 0.004$). Significant negative correlations were found between concentrations of SAH in plasma and platelet APP and between SAM and platelet α -synuclein. A larger echogenic area of the substantia nigra was related to higher serum concentrations of MMA ($P = 0.016$).

CONCLUSIONS: Markers of neurodegeneration (APP, α -synuclein) are related to markers of methylation (SAM, SAH) in patients with PD. Better cognitive

function was related to higher methylation potential (SAM/SAH ratio).

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After Alzheimer disease (AD),³ Parkinson disease (PD) is the second most common neurodegenerative disease. The loss of dopaminergic neurons in the substantia nigra region of the brain is 1 pathological hallmark in patients with PD. The second hallmark of the disease is the presence of intracellular inclusions—Lewy bodies and Lewy neurons that are rich in α -synuclein, an abundant synaptic terminal protein (1).

Increased concentrations of plasma total homocysteine (tHcy) have been reported in patients with PD (2, 3). The increase in tHcy in patients with PD depends mainly on folate and vitamin B₁₂ status and the treatment used to manage PD symptoms. Increased tHcy has been associated with worse cognition (4), sensory neuropathy (5), and depression (6) in patients with PD. Hyperhomocysteinemia (HHCY) is very common in PD patients treated with L-3,4-dihydroxyphenylalanine (L-dopa). L-dopa is methylated by catechol-O-methyltransferase (COMT), an S-adenosyl methionine (SAM)-dependent enzyme. A significant increase in concentration of tHcy occurs in PD patients after starting L-dopa treatment (7). Animal studies have shown that L-dopa causes depletion of cerebral SAM (between 36% and 76%) and a marked increase in S-adenosyl homocysteine (SAH) concentrations (8). Higher demands for SAM in L-dopa-treated patients in the face of unchanged food intake of methyl group donors (methionine, choline) might require greater de novo synthesis of endogenous SAM.

On the one hand, AD is associated with an abnormal accumulation of amyloid- β . In AD, amyloid- β

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³ Nonstandard abbreviations: AD, Alzheimer disease; PD, Parkinson disease; tHcy, total homocysteine; HHCY, hyperhomocysteinemia; L-dopa, L-3,4-dihydroxyphenylalanine; COMT, catechol-O-methyltransferase; SAM, S-adenosyl methionine; SAH, S-adenosyl homocysteine; APP, amyloid- β precursor protein; ER, endoplasmic reticulum; MMA, methylmalonic acid; UPDRS, Unified Parkinson's Disease Rating Scale; MMSE, Mini-Mental Status Examination; PLP, pyridoxal-5-phosphate; holoTC, holotranscobalamin; MAO, monoamine oxidase; PCK, protein kinase C; PIMT, isoaspartyl methyl transferase; MAP, mitogen-activated protein; UPS, ubiquitin proteasome system.

produced from amyloid- β precursor protein (APP) accumulates in the intracellular and the extracellular space, leading to amyloid plaques. On the other hand, PD is characterized by accumulation of α -synuclein. α -Synuclein is a 140-amino acid peptide that is expressed in the presynaptic terminals of neuronal cells. The exact physiological role of α -synuclein is not known, but it can be assumed that this protein modulates neurotransmitter release, participates in endoplasmic reticulum (ER)/Golgi trafficking (9), or is loosely associated with synaptic vesicles. Approximately 15%–25% of AD patients develop motor deficits with α -synuclein-rich Lewy body-like inclusions (10). A direct interaction between amyloid- β and α -synuclein in the brains of patients with Lewy bodies has recently been reported (11). Lewy bodies contain APP (11). The occurrence of amyloid- β and α -synuclein in Lewy bodies suggests that their accumulation might be related to mutual pathologic conditions.

An increased number of endothelial cell nuclei in the substantia nigra pars compacta has been reported (12). Moreover, the changes in vascular density might participate in neuronal vulnerability to toxins, such as homocysteine and methylmalonic acid (MMA). We hypothesized that the metabolic condition in patients with PD can promote neurodegeneration, probably by enhancing accumulation of some functional proteins, such as α -synuclein and β -amyloid. To test this hypothesis, we measured plasma methylation markers, SAM and SAH, in relation to plasma β -amyloid(1–42) concentrations and APP and α -synuclein isolated from platelets. Moreover, we investigated the association between the substantia nigra echogenic area and blood parameters.

Participants and Methods

The study included 87 patients with Parkinson disease (median age 68 years; 35 men). Patients were recruited from the Movement Disorders Centre at the Department of Neurology, University Hospital of Saarland. Informed consent was obtained from all study participants. The study was approved by the local Ethical Committee at the University Hospital of Saarland.

We measured the physical status of each patient using the Unified Parkinson's Disease Rating Scale (UPDRS) part III (the motor scale) and global cognitive functioning using the Mini-Mental Status Examination (MMSE) and DemTect scores. Exclusion criteria were renal dysfunction defined as serum creatinine $>106.1 \mu\text{mol/L}$, B vitamin supplementation, or a recent cerebral or coronary event (in the last 3 months). Information about age, duration of PD, smoking, diabetes, depression, dementia, and PD medications was gathered.

Nonfasting blood samples were drawn into a plain tube and EDTA-anticoagulant tubes. Within 30 min, the blood was centrifuged at 2000g for 10 min. EDTA plasma (500 μL) was removed and treated with 50 μL of 1N acetic acid, mixed, and stored at -80°C for SAM and SAH assays. In addition, we collected citrate-anticoagulated blood from a subset of 45 patients for platelet isolation. Citrate blood was immediately centrifuged at 200g for 10 min. Platelet-rich plasma was aspirated; the platelets were washed twice with cold PBS, and the washed platelets were immediately frozen at -80°C before analyses of APP and α -synuclein.

We prepared platelet extracts by lysing the cells with 500 μL extraction buffer (10 mmol Tris, pH 7.4, 100 mmol NaCl, 1 mmol EDTA, 1 mmol EGTA, 1 mmol NaF, 20 mmol $\text{Na}_4\text{P}_2\text{O}_7$, 2 mmol Na_3VO_4 , 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate, 1 mmol/L phenylmethylsulfonyl fluoride) plus a protease inhibitor cocktail (Roche Diagnostics). The platelet extract was sonicated 5 times on ice for 10 s with short intervals. We measured the concentration of total protein in the cell extracts using bicinchoninic acid solution (Sigma) plus 4% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and used BSA for the calibration curve. We measured concentrations of APP and α -synuclein in platelet extracts using reagents from Biomol and BioSource, respectively. The capture antibody for APP assay recognizes the N-terminal part of human APP, and the detection antibody recognizes the N-terminal part of amyloid- β peptide. Thus, this ELISA detects soluble APPs α (the nonamyloidogenic APP) APP770, 751 (soluble APP), APP733, and APP695. The APP695 is found predominantly in brain tissues (13), whereas APP770 and APP751 isoforms are abundant in platelets (14). The kit for detection of α -synuclein recognizes total α -synuclein independent of its phosphorylation status. Platelet markers were available from a subset of 45 patients. We adjusted the platelet markers for total protein measured in an aliquot of the cell extracts.

We measured concentrations of amyloid- β (1–42) in serum using a high-sensitivity ELISA test from Innogenetics and serum concentrations of tHcy, cystathionine, and MMA using GC-MS with isotope-labeled internal standards (15). We measured concentrations of SAM and SAH in EDTA-plasma using LC-MS/MS, applying a slightly modified method according to Gellekink et al. (16) and concentrations of total vitamin B_{12} and folate in serum using a chemiluminescence immunoassay (Advia Centaur System). We measured plasma vitamin B_6 [pyridoxal-5-phosphate (PLP)] by HPLC connected with a fluorescence detector using reagents from Immundiagnostik and serum holotranscobalamin (holoTC) using an automatic method on AxSYM.

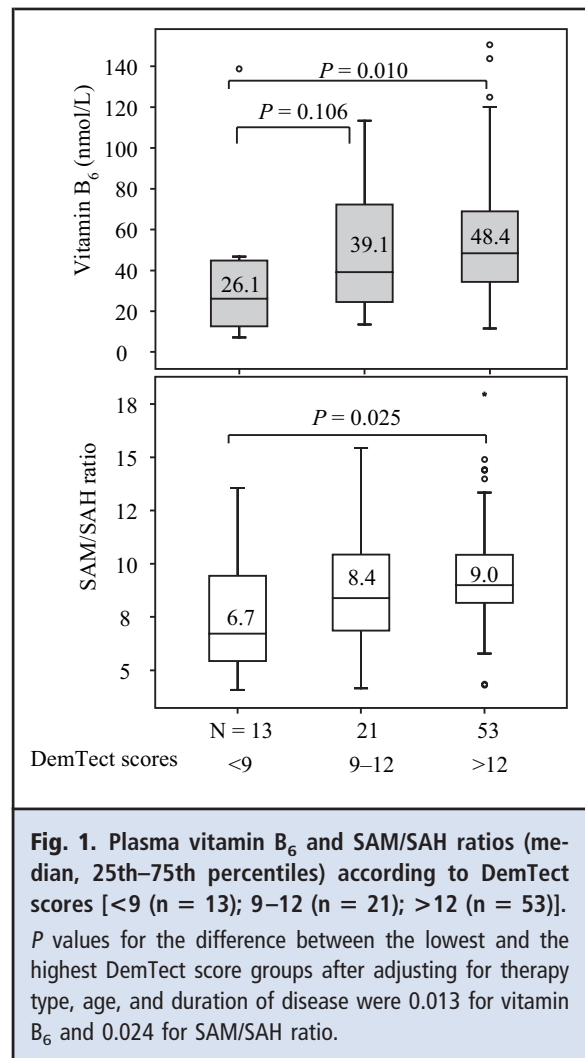
We used brain parenchyma sonography, an ultrasound technique, to display the tissue echogenicity of the substantia nigra in 59 patients. This method proved to be reliable and diagnostically sensitive in detecting degeneration of the substantia nigra in patients with Parkinson disease (17). Because this variable is semiquantitative, we stratified the patients into 3 groups: patients with substantia nigra echogenic area $>0.22 \text{ cm}^2$ ($n = 27$), those with substantia nigra echogenic area $\leq 0.22 \text{ cm}^2$ ($n = 24$), and patients with inconclusive findings ($n = 8$). The last group included patients whose insufficient temporal bone windows on both sides made the measurement of the substantia nigra not possible. None of the patients were taking B vitamins at the time of recruitment.

We conducted statistical analyses using SPSS version 15.0. Continuous variables were expressed as median (10th–90th) percentiles. We compared differences between means by ANOVA and the post hoc Tamhane tests and conducted adjustments for covariates using univariate analyses. The data were log-transformed before applying tests that require normal distribution of the data. We evaluated correlations between different variables using the Spearman test. Three groups of DemTect scores [<9 ($n = 13$); $9\text{--}12$ ($n = 21$); >12 ($n = 53$)] were used in Fig. 1. We applied stepwise backward regression analyses for investigating independent variables that might predict variations in DemTect scores and SAM/SAH ratios between the patients. P values <0.05 were considered statistically significant.

Results

Patients' main characteristics and associated medications are shown in Supplemental Table 1, which accompanies the online version of this article at www.clinchem.org/content/vol55/issue10. We found no effect of smoking, antidepressives, monoamine oxidase (MAO) inhibitors, or diabetes on concentrations of tHcy or related metabolic markers (data not shown).

Table 1 shows the concentrations of measured biochemical markers in the total group of PD patients and according to the type of PD treatment. Patients receiving a dopamine agonist were younger than those receiving L-dopa and those receiving a combination of an agonist plus L-dopa (median age 61 vs 74 and 69 years, respectively). Moreover, the shortest treatment duration for PD was in dopamine agonist-treated patients, and the longest was in those receiving an agonist plus L-dopa (median duration 2.5 vs 8.0 years). The median concentration of plasma SAH was highest in the L-dopa single treatment group and lowest in the agonist single treatment (median 17.2 vs 12.7 nmol/L). The highest concentrations of SAH, MMA, and tHcy and the lowest



concentrations of holoTC and SAM/SAH ratio in L-dopa-treated patients were related to the higher age of the patients treated with L-dopa (median 74 years) and the duration of the disease. The between-group differences in concentrations of SAH, MMA, tHcy, and SAM/SAH disappeared after adjusting for age and duration of disease ($P > 0.05$). Participants receiving L-dopa alone or in combination with COMT-inhibitor had comparable concentrations of the biomarkers tHcy, MMA, SAH, SAM, SAM/SAH ratios, and the vitamins (B₆, B₁₂, holoTC, and folate) (data not shown).

As shown in Fig. 1, patients with PD who had normal cognitive function (DemTect scores >12) had significantly higher concentrations of plasma vitamin B₆ and SAM/SAH ratios (median vitamin B₆ 48.4 nmol/L and SAM/SAH 9.0) compared with patients with DemTect scores <9 (median vitamin B₆ 26.1 nmol/L and SAM/SAH 6.7). Patients with mild cognitive decline (DemTect scores $9\text{--}12$) had intermediate vitamin

Table 1. Markers estimated in the total group of PD patients and according to treatment.^a

Markers	All	Dopamine agonist	L-dopa	L-dopa plus dopamine agonist	P (ANOVA) ^b
n	87	28	20	35	
Age, years	68 (54–78)	61 (52–69)	74 (62–80) ^c	69 (56–79) ^c	<0.001
PD duration, years	5 (0–12)	2.5 (0–10)	4 (0–15)	8 (3–15) ^c	<0.001
DemTect scores	14 (7–18)	15.5 (10.9–18.0)	13.0 (3.0–18.0)	12.5 (7.0–17.5)	0.092
tHcy, $\mu\text{mol/L}$	14.8 (9.4–23.2)	13.3 (7.6–19.3)	16.4 (11.0–23.5) ^c	15.4 (10.5–24.6)	0.047
Cys, nmol/L	260 (158–693)	238 (162–744)	234 (157–1134)	277 (144–802)	0.780
MMA, nmol/L	261 (170–884)	218 (148–406)	337 (196–815) ^c	283 (170–1179)	0.032
Folate, nmol/L	19.0 (8.9–38.1)	20.7 (10.8–42.4)	21.2 (12.7–45.9)	15.7 (7.8–32.3)	0.094
Vitamin B ₁₂ , pmol/L	236 (152–355)	253 (148–321)	239 (152–470)	235 (149–384)	0.932
HoloTC, pmol/L	43 (22–96)	44 (23–78)	35 (20–192)	48 (23–97)	0.867
Vitamin B ₆ , nmol/L	43.7 (15.7–121.0)	43.7 (15.7–121.0)	49.5 (11.1–119.4)	34.0 (10.8–149.7) ^c	0.019
SAH, nmol/L	13.6 (9.8–28.3)	13.6 (9.8–28.3)	17.2 (11.3–32.9) ^c	13.7 (10.0–26.2)	0.005
SAM, nmol/L	124 (93–173)	124 (93–173)	133 (96–204)	122 (87–168)	0.394
SAM/SAH ratio	8.7 (5.5–13.3)	8.7 (5.5–13.3)	7.8 (5.5–11.4) ^c	8.4 (5.4–11.2) ^c	0.005
Substantia nigra echogenic area, cm ²	0.23 (0.13–0.48)	0.19 (0.12–0.36)	0.34 (0.10–0.63)	0.26 (0.13–0.50)	0.079
Platelet α -synuclein, $\mu\text{g/L}$	20.5 (11.8–36.1)	25.1 (14.3–57.5)	20.6 (10.4–33.0)	20.4 (12.3–32.6)	0.173
Platelet APP, $\mu\text{g/L}$	700 (48–31340)	595 (17–1374)	551 (190–1360)	741 (0–1418)	0.289
Plasma amyloid- β , ng/L	26.9 (24.0–29.6)	25.8 (23.7–28.7)	25.7 (23.5–31.1)	27.8 (24.8–29.7)	0.705
Creatinine, $\mu\text{mol/L}$	88.4 (61.9–114.9)	79.6 (61.9–99.0)	84.0 (61.9–132.6)	88.4 (61.9–112.3)	0.180

^a Data are median (10th–90th percentile).
^b P values after adjustment for age and duration of disease were for tHcy ($P = 0.970$), MMA ($P = 0.889$), vitamin B₆ ($P = 0.196$), SAH ($P = 0.164$), and SAM/SAH ($P = 0.426$). Platelet markers were adjusted for total cellular proteins. Four patients received neither L-dopa nor dopamine agonists.
^c $P < 0.05$ compared with the group that received dopamine agonists.

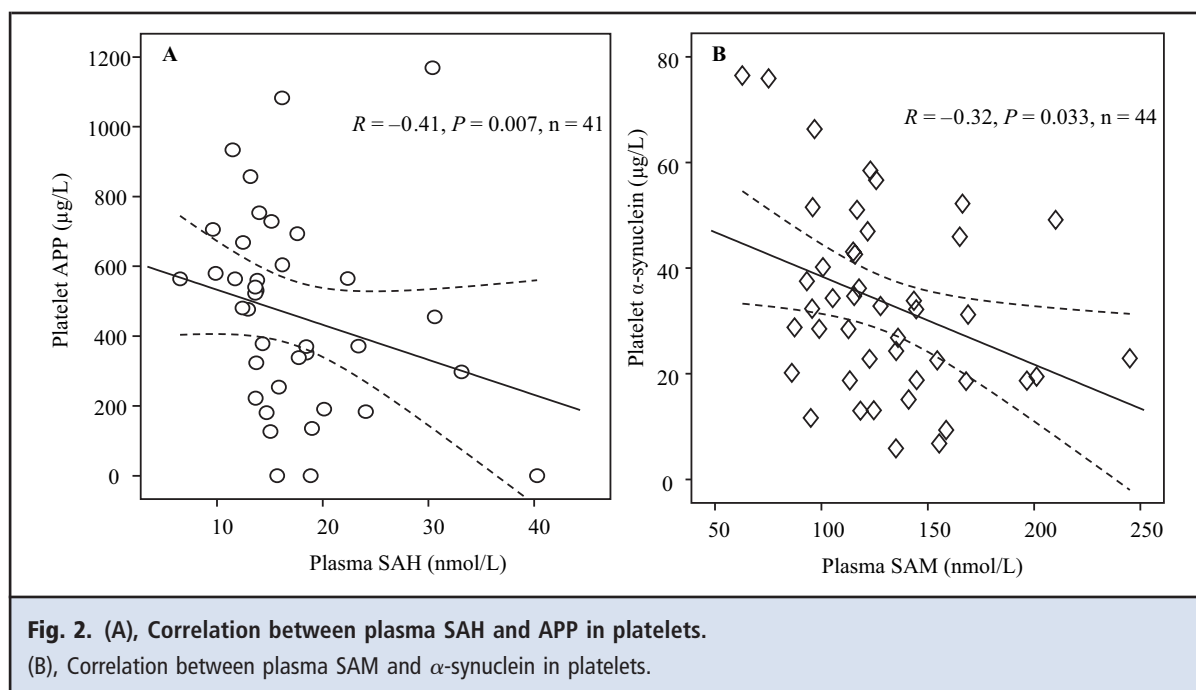
B₆ and SAM/SAH ratios. After adjusting for therapy type, age, and duration of disease, the difference between the lowest and the highest DemTect score groups remained significant for vitamin B₆ ($P = 0.013$) and SAM/SAH ratio ($P = 0.024$). Moreover, DemTect scores were negatively related to plasma amyloid- β (1–42) (Spearman correlation coefficient $r = -0.45$, $P < 0.001$).

We applied stepwise backward regression analyses to determine factors that might predict DemTect scores in the study patients (online Supplemental Table 2, model 1). Of all factors included in this analysis, only SAM/SAH ratio ($\beta = 0.612$, $P = 0.004$) was a significant predictor of DemTect scores. Significant predictors of plasma SAM/SAH ratio (model 2) were concentrations of serum tHcy ($\beta = -0.276$, $P < 0.001$) and serum creatinine ($\beta = -0.422$, $P = 0.001$) (online Supplemental Table 2).

The plasma SAH concentration was negatively related to platelet APP (Spearman correlation coefficient $r = -0.41$, $P = 0.007$) (Fig. 2A), and plasma SAM was negatively related to α -synuclein in platelets ($r =$

-0.32 , $P = 0.033$) (Fig. 2B). Additionally, platelet APP and plasma amyloid- β (1–42) were negatively correlated ($r = -0.34$, $P = 0.028$). APP and α -synuclein were also negatively related ($r = -0.35$, $P = 0.031$). In contrast, plasma concentrations of amyloid- β (1–42) correlated positively with the concentrations of α -synuclein in platelets ($r = 0.42$, $P = 0.005$). A correlation was found between plasma concentrations of SAM and SAH ($r = 0.63$, $P < 0.001$). Moreover, concentrations of folate and vitamin B₆ showed also a positive correlation ($r = 0.60$, $P < 0.001$).

Fig. 3 shows concentrations of folate, vitamin B₆, tHcy, SAH, and SAM/SAH ratios according to tertiles of plasma amyloid- β (1–42). Tertiles of amyloid- β (<25.8, 25.8–27.8, >27.8 ng/L) were used in Fig. 3. Patients in the highest tertile of plasma amyloid- β (1–42) had significantly higher concentrations of tHcy and SAH and lower concentrations of folate, vitamin B₆, and SAM/SAH ratio compared to patients in the lowest tertile of amyloid- β (1–42). However, patients in the highest tertile of amyloid- β were older (median age 72 vs 63 years), had PD for a longer duration (median



6.0 vs 3.5 years), and were therefore more likely to receive combined treatment of L-dopa plus dopamine agonist compared to those in the lowest tertile. After adjusting for age, disease duration, and type of treatment, the difference in folate ($P = 0.005$) and vitamin B₆ ($P = 0.014$) between the lowest and the highest amyloid- β tertiles remained significant, tHcy tended to be lower in the lowest tertile ($P = 0.078$), and SAH and SAM/SAH ratio were no longer significant ($P = 0.114$ for SAH and $P = 0.103$ for SAM/SAH ratio).

Table 2 shows blood markers in relation to substantia nigra echogenic area. Higher substantia nigra echogenic area was associated with higher plasma concentrations of MMA (236 vs 351 nmol/L, $P = 0.016$), but not with other markers of vitamin B₁₂ status (total vitamin B₁₂ and holoTC), tHcy, or markers of methylation. The difference in MMA between the 2 groups remained significant after adjusting for creatinine and treatment type ($P = 0.036$).

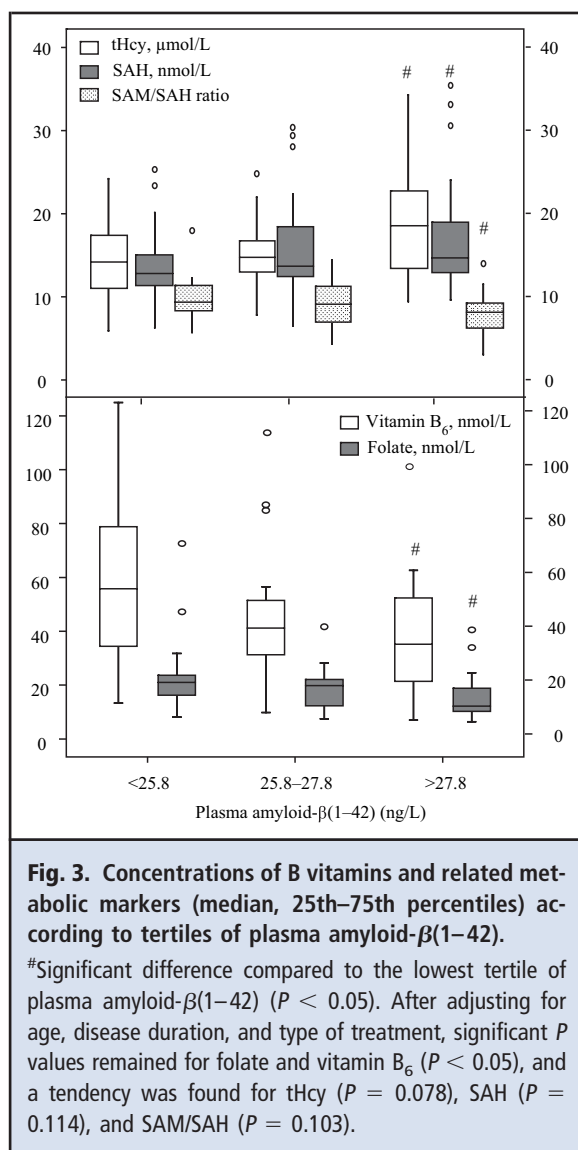
Discussion

AD and PD are the leading neurodegenerative diseases in elderly people resulting in dementia and movement disorders, respectively. Lewy bodies with α -synuclein-rich inclusions characterize PD brains. Moreover, Parkinson patients might develop dementia and amyloid- β -rich plaques. These overlapping disorders suggest that amyloid- β and α -synuclein might be associated (18). The occurrence of amyloid- β and α -synuclein in Lewy bodies also suggests that their accumulation

might be related to mutual pathologic conditions. Hypomethylation might participate to neurodegeneration, probably by increasing the expression of APP or its hydrolysis to amyloid- β (19). In this study, we showed an association between concentrations of neurodegenerative proteins (APP and α -synuclein) and that of the methyl donor, SAM, or the methyltransferase inhibitor, SAH.

Concentrations of tHcy in patients with PD from this study were similar to those reported in previous studies (3). The effect of treatment type on the metabolic markers in this study could not be confirmed. On the one hand, concentrations of tHcy, MMA, and SAH were higher in patients receiving single treatment with L-dopa compared to the other treatment groups. However, these differences were probably related to age differences between the treatment groups (Table 1). On the other hand, we found no significant role of COMT inhibitors in sparing SAM or lowering tHcy in L-dopa-treated patients. Results concerning the role of COMT inhibitors in preventing L-dopa-induced HHcy are conflicting (20–22), suggesting that this effect might be related to other factors such as the basal vitamin status.

The concentration of tHcy was a significant predictor of the methylation index in plasma (SAM/SAH ratio). Additionally, the positive correlation between plasma concentrations of SAM and SAH ($r = 0.63$) suggests that the increased SAM was secondary to the SAH increase, which is in turn related to ineffective tHcy catabolism. Importantly, the SAM/SAH ratio was



a significant positive predictor of DemTect scores in patients with Parkinson disease (online Supplemental Table 2). The association between cognitive function scores and plasma SAM/SAH ratio remained significant after adjusting for age, disease duration, and type of treatment, thus suggesting a role for methylation in some neurodegenerative processes (Fig. 4).

The positive association between DemTect score and plasma concentration of vitamin B_6 was independent of age, duration, and type of PD treatment, suggesting that better vitamin B_6 might delay cognitive decline in PD patients. Glutathione is a major antioxidant in dopaminergic neurons of PD patients. Moreover, oxidative stress is related to the degenerative process in Parkinson disease (23). Vitamin B_6 enhances the direct flow of tHcy via cystathionine β -synthase

and cystathionine γ -lyase into cysteine, the precursor of glutathione (24). Vitamin B_6 deficiency induces oxidative stress, which in turn affects several enzymes in 1-carbon metabolism, thus indirectly causing lower glutathione (25). In addition, vitamin B_6 is a cofactor for serine hydroxymethyl transferase, a folate catabolizing enzyme. This implies that vitamin B_6 also indirectly enhances tHcy removal by making folate available for Hcy remethylation. The last suggestion is in accordance with the direct correlation we found between folate and vitamin B_6 ($r = 0.60$, $P < 0.001$). A possible protective role of vitamin B_6 and folate in PD patients is supported by data showing negative associations between vitamin B_6 or folate and amyloid- $\beta(1-42)$ (Fig. 3). Taken together, these data suggest that vitamin B_6 might protect the brain from amyloid- β accumulation, oxidative stress, and dopamine deficiency, thus delaying a decline in cognitive ability.

Plasma concentrations of amyloid- $\beta(1-42)$ are increased in patients with neurodegenerative diseases (26). This protein is thought to be produced from APP in the neurons and to cross the blood–brain barrier to the blood. Several features of neurons resemble those of platelets (27), such as expressing APP (28) and producing amyloid- β (29). Therefore, studying brain proteins that are expressed in platelets represents an interesting approach. APP can be hydrolysed via the β -pathway by means of β -secretases and γ -secretases (Presenilin I), producing sAPP β and amyloid- β , respectively. The alternative nonamyloidogenic pathway is mediated by α and δ secretases and produces sAPP α and P3. Interestingly, the negative correlation between plasma concentration of the methyl inhibitor, SAH, and platelet-APP (Fig. 2) supports the hypothesis that SAH might enhance the amyloidogenic pathway and thereby hydrolysis of APP to amyloid- $\beta(1-42)$. Hypomethylation might be related to enhanced production of amyloid- β via increasing the expression of Presenilin I gene (30). The inverse correlation between plasma amyloid- $\beta(1-42)$ and nonamyloidogenic APP in the platelets ($r = -0.34$, $P = 0.028$) suggests that more amyloid- $\beta(1-42)$ is formed under metabolic conditions supporting the amyloidogenic pathway, such as increased SAH. One possible mechanism is disturbed phospholipid metabolism in the case of hypomethylation. Low phosphatidylcholine, the methylated product of phosphatidylethanolamine, can cause low protein kinase C (PKC), thus leading to enhanced conversion of APP to amyloid- β (31) (Fig. 4).

α -Synuclein is an important protein that participates in neurodegeneration in PD patients. α -Synuclein is involved in dopaminergic neurotransmission. This protein regulates dopamine biosynthesis and homeostasis (32). α -Synuclein is expressed in

Table 2. Variables in relation to substantia nigra echogenic area.^a

Marker	Substantia nigra echogenic area ≤ 0.22 cm ²	Substantia nigra echogenic area > 0.22 cm ²	P (ANOVA)
n	24	27	
Age, years	63 (9.0)	67 (8.3)	0.123
Duration, years	4.5 (4.4)	6.0 (7.4)	0.120
DemTect score	14 (3)	14 (5)	0.246
Creatinine, μ mol/L	81.6 (11.3)	91.8 (21.6)	0.099
tHcy, μ mol/L	14.0 (3.2)	15.8 (10.8)	0.207
MMA, nmol/L ^b	236 (107)	351 (544)	0.016
Folate, nmol/L	19.2 (16.1)	17.9 (13.1)	0.614
HoloTC, pmol/L	47 (19)	48 (73)	0.676
Vitamin B ₁₂ , pmol/L	232 (70)	251 (102)	0.385
Vitamin B ₆ , nmol/L	51 (104)	46 (46)	0.616
SAM/SAH ratio	9.2 (2.1)	8.4 (2.5)	0.249
L-dopa plus dopamine agonist, n (%)	8 (33)	13 (48)	
Dopamine agonist, n (%)	14 (58)	5 (18.5)	
L-dopa, n (%)	2 (8)	9 (33)	

^a Data are geometric mean (SD) unless otherwise mentioned.
^b P = 0.036 after adjusting for serum creatinine.

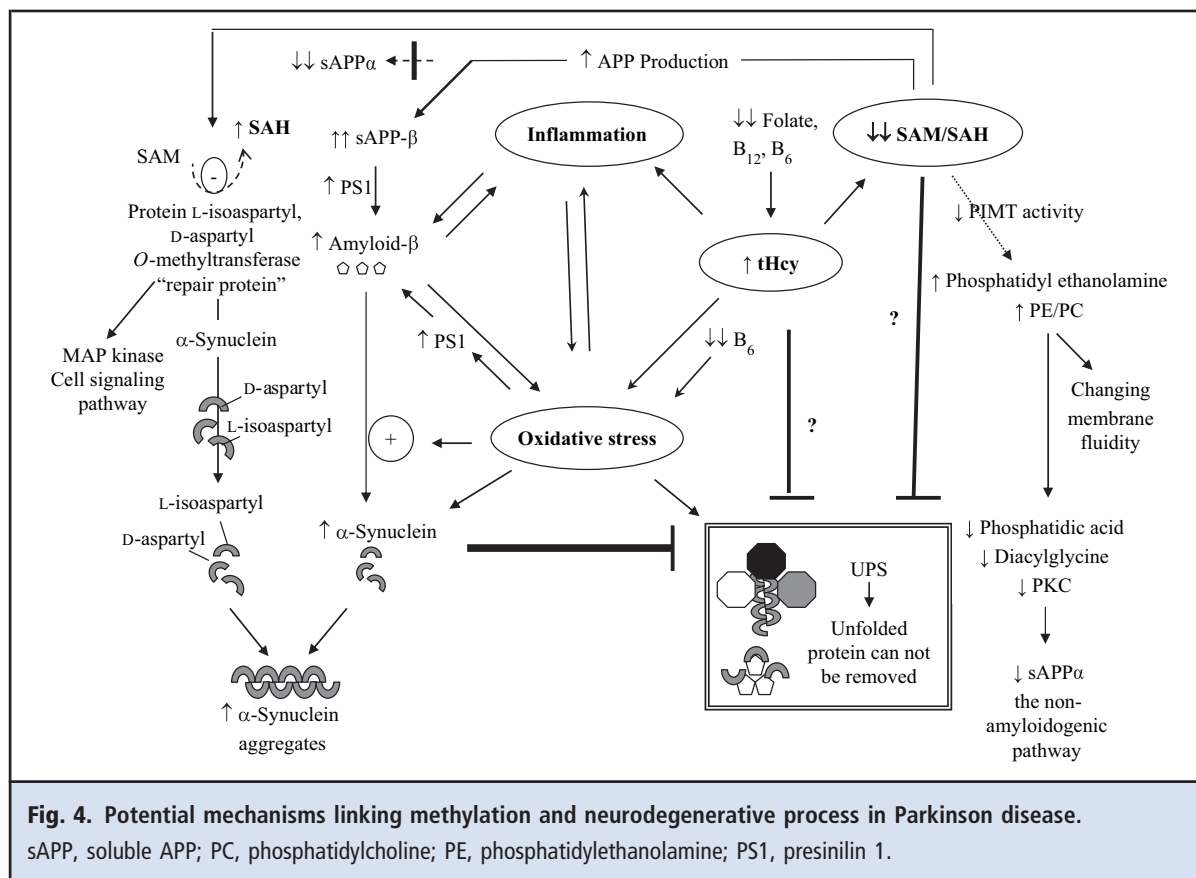
platelets. The inverse correlation between plasma concentrations of SAM and platelet α -synuclein is an interesting finding in this study (Fig. 2). Two possible explanations exist. First, a limited availability of SAM might enhance expression of α -synuclein gene. HHCY has been related to DNA hypomethylation (33). An increased mRNA for α -synuclein has been found in chronic alcoholism (34), where disorders of Hcy and methylation status are very common (35). Second, SAH can inhibit isoaspartyl methyltransferase (PIMT), an SAM-dependent enzyme responsible for initiating the repair of isoaspartyl residues in aged or stress-damaged proteins including α -synuclein (36). PIMT is expressed in platelets (37). In an animal model of methyl group deficiency, lower SAM was associated with enhanced retention of isoaspartyl-rich α -synuclein, which tended to aggregate and cause aberrant synaptic transmission in the deficient animals (36). PIMT-knockdown cells showed hyperphosphorylation of components of the mitogen-activated protein (MAP) kinase cascade compared to control cells (38). MAP kinase component might be related to expression of α -synuclein (39).

α -Synuclein and amyloid- β can form co-oligomers on the neuronal cell membrane (11). Amyloid interaction with α -synuclein facilitates aggregation of α -synuclein. This pore-forming complex enhances accumulation of calcium within the cells. The positive correlation between plasma amyloid- β (1–42) and platelet α -synuclein ($r = 0.42$) in our study suggests a

mutual mechanism controlling either the production of both proteins or their catabolism. One plausible mechanism might be the ubiquitin proteasome system (UPS) that binds and eliminates aggregated proteins. HHCY in PD patients causes oxidative stress and can thus exhaust the UPS leading to amyloid- β and α -synuclein oligomers (Fig. 4).

Higher concentrations of serum MMA were found in patients with a larger substantia nigra echogenic area compared to those with a smaller area. This association could not be explained by vitamin B₁₂ status, because neither holoTC nor total B₁₂ was related to the area of the substantia nigra. Serum creatinine also was not responsible for the difference in MMA concentrations according to the area of the substantia nigra. MMA might enhance the phosphorylation of cytoskeletal proteins in the cerebral cortex of animals (40). Moreover, MMA has been shown to interfere with the Krebs cycle and the mitochondrial electron transport system, causing neural cell death. These results suggest that at least part of the neurotoxicity of vitamin B₁₂ deficiency is related to MMA accumulation, and not to the low vitamin concentration.

Taking these data together, we found that better cognitive function in patients with Parkinson disease was related to a higher methylation potential (SAM/SAH ratio) and higher plasma vitamin B₆. The effect of vitamin B₆ on cognitive function is probably indirectly related to improving methylation status and reducing the production of amyloid- β . Hypomethylation (high



SAH, or low SAM) might enhance APP degradation via the amyloidogenic pathway and increase α -synuclein. Future studies might test the effect of vitamins B or SAM supplementation on neurodegenerative markers in patients with Parkinson disease.

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