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Folate and Methylation Status in Relation to Phosphorylated Tau Protein$_{(181P)}$ and β-Amyloid(1–42) in Cerebrospinal Fluid

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Background: **Increased plasma total homocysteine (tHcy) is a risk factor for neurological diseases, but the underlying pathophysiology has not been adequately explained.**

Methods: **We evaluated concentrations of tHcy, S-adenosyl homocysteine (SAH), S-adenosyl methionine (SAM), folate, and vitamin B_{12} in cerebrospinal fluid (CSF) and plasma or serum from 182 patients with different neurological disorders. We measured concentrations of phosphorylated tau protein (P-tau)$_{(181P)}$ and β-amyloid(1–42) in the CSF.**

Results: **Aging was associated with higher concentrations of tHcy and SAH in the CSF, in addition to lower concentrations of CSF folate and lower SAM:SAH ratio. Concentrations of CSF SAH and CSF folate correlated significantly with those of P-tau (r = 0.46 and r = −0.28, respectively). Moreover, P-tau correlated negatively with SAM:SAH ratio (r = −0.40, P <0.001). The association between SAH and higher P-tau was observed in 3 age groups (<41, 41–60, and >60 years). CSF tHcy was predicted by concentrations of CSF cystathionine (β = 0.478), folate (β = −0.403), albumin (β = 0.349), and age (β = 0.298).**

Conclusions: **tHcy concentration in the brain is related to age, B vitamins, and CSF albumin. Increase of CSF SAH is related to increased CSF P-tau; decreased degradation of P-tau might be a plausible explanation. Disturbed methyl group metabolism may be the link between hyperhomocysteinemia and neurodegeneration. Lowering tHcy and SAH might protect the brain by preventing P-tau accumulation.**

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The importance of methyl group metabolism for brain function has been recognized *(1)*. S-adenosyl methionine (SAM)[3] is generated from methionine metabolism and constitutes a very important methyl donor in the central nervous system. In a 1st step, methionine is activated by ATP to form SAM. Subsequently, SAM is converted into S-adenosyl homocysteine (SAH) after donating its methyl group to other cellular components. SAH, a potent competitive inhibitor for several methyl transferases, binds to the enzymes with a greater affinity than SAM *(2)*. SAH is further hydrolyzed into homocysteine (Hcy) that can be either remethylated into methionine or transsulfurated to cysteine. Alternatively, Hcy can be converted via SAH-hydrolase into SAH in a reversible reaction that favors the formation of SAH when concentrations of total Hcy (tHcy) increase. SAM-dependent methylation encompasses a wide range of biological reactions, including myelin synthesis; synthesis and catabolism of neurotransmitters and phospholipids; and methylation of proteins, RNA, and DNA *(3)*. Several neurological disorders have been related to abnormalities in methyl group or 1-carbon metabolisms *(4, 5)*.

In addition to its major role as a methyl donor, SAM represents a metabolic link between the methylation and the transsulfuration pathways *(6)*. SAM enhances the activity of cystathionine β synthase, and thereby the flux

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[3] Nonstandard abbreviations: SAM, S-adenosyl methionine; SAH, S-adenosylhomocysteine; Hcy, homocysteine; tHcy, total homocysteine; P-tau, hyperphosphorylated tau protein; CSF, cerebrospinal fluid; PP2A, protein phosphatase 2A; Cys, cystathionine; MMA, methylmalonic acid; holoTC, holotranscobalamin.

of Hcy via the transsulfuration pathway, to produce more cysteine, the precursor of glutathione. Because folate (5-methyltetrahydrofolate) and vitamin B_{12} (methyl cobalamin) play important roles in recycling Hcy to methionine, these 2 vitamins are considered essential for the availability of methyl groups in the brain. Substantial evidence suggests that the alternative remethylation pathway of Hcy via betaine-Hcy methyltransferase does not account for Hcy remethylation in the brain *(7)*.

Increased plasma tHcy is an independent risk factor for neurodegenerative and psychiatric disorders *(5, 8–13)*. A tHcy concentration >10 μmol/L in plasma has been associated with brain atrophy in elderly people *(14)*. Moreover, longitudinal studies demonstrated an association between baseline plasma tHcy concentrations and some measures of cognitive or memory decline after several years of follow-up *(15, 16)*. Increased plasma tHcy is associated with increased concentrations of tHcy and SAH in the brain *(17)*. This may explain the link between hyperhomocysteinemia and neural damage, but the underlying pathophysiology has not been adequately explained.

Neurodegenerative diseases share a common feature, accumulation of misfolded proteins. Tau and β-amyloid are 2 examples of proteins that accumulate in brains of patients with dementia. Concentrations of β-amyloid(1–42) and hyperphosphorylated tau protein $(P\text{-tau})_{(181P)}$ in cerebrospinal fluid (CSF) predict the development of dementia *(18)*. A positive association between plasma concentrations of tHcy and β-amyloid(1–40) and β-amyloid(1–42) has been reported in neurodegenerative disease *(11)*. Moreover, tHcy can increase the vulnerability of neurons to being damaged by β-amyloid *(19)*. Likewise, hyperphosphorylation of tau influences its structure and association with the plasma membrane. Increased P-tau may be related to a lower phosphatase activity or to increased activity of kinases (see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue5). Because the activation of protein phosphatase 2A (PP2A) is SAM dependent, a possible link between dementia and reduced methylation has been hypothesized *(20)*; this association has never been investigated in human studies. Data about the distributions and determinants of biomarkers of B vitamins and methylation status in blood and CSF are limited. We investigated the relationship of markers of neurodegeneration, methylation, and vitamin status in CSF samples from patients with neurological disorders.

PATIENTS AND METHODS

The study included 182 patients who were treated at the Department of Neurology, University Hospital of the Saarland, from April 2002 to April 2004: 31 patients with multiple sclerosis, 19 patients with stroke, 31 patients with dementia (8 with Alzheimer disease), 36 patients with peripheral neuropathy, and a control group of 65 patients with various neurological diseases other than dementia, Parkinson disease, polyneuropathy, multiple sclerosis, stroke, Alzheimer disease, or depression. The diagnosis of Alzheimer disease was by criteria of a cognitive subscale score on the Alzheimer's Disease Assessment Scale and Mini Mental Examination Scores. The control group has been described *(21)*. Exclusion criteria included renal or liver dysfunction and alcoholism.

We collected nonfasting blood samples from all patients; serum and EDTA plasma were available, and CSF samples were obtained during clinically indicated lumbar punctures. Blood and CSF samples collected within 24 h of each other were directly centrifuged and stored at −80 °C until analysis. Aliquots of the EDTA plasma and CSF were immediately deproteinized using perchloric acid (10%). These samples were stored at −80 °C and used for SAM and SAH assays. CSF samples contaminated with peripheral blood or hemoglobin were not included in the study. The study was approved by the Ethics Committee at the Saarland University Hospital, and all patients gave written informed consent.

We measured concentrations of SAM and SAH by use of a modified liquid chromatography–tandem mass spectrometry method according to Gellekink et al. *(22)*. The CVs for SAM and SAH assays were 4.8% and 8%, respectively, at concentrations of 103 nmol/L for SAM and 15.6 nmol/L for SAH. We measured concentrations of tHcy, cystathionine (Cys), and methylmalonic acid (MMA) in serum and CSF samples by use of gas chromatography–mass spectrometry as described *(23)*. The CV for tHcy was <5% (at 8.0 and 16.0 μmol/L) in serum and <10% in CSF (at 0.30 μmol/L). The CV for MMA was <6% in serum and CSF (at 290 and 540 nmol/L, respectively), and the CV for cystathionine was <8% in serum (at 300 nmol/L) and <10% in CSF (at 60 nmol/L). We used pool serum and pool CSF prepared in-house on each run with the study samples to calculate CV.

We measured the concentrations of total vitamin B_{12} and folate in serum and CSF by use of a chemiluminescence immunoassay (ADVIA Centaur System); plasma concentrations of vitamin B_6 (pyridoxal-5-phosphate, PLP) by use of HPLC connected with fluorescence detector using reagents from Immundiagnostik; concentrations of holotranscobalamin (holoTC) in serum and CSF by use of an RIA from Axis-Shield *(24)*; concentrations in the CSF of $P\text{-tau}_{(181P)}$ and β-amyloid(1–42) by use of specific monoclonal antibodies against these 2 proteins (Innogenetics); and serum concentrations of creatinine, cholesterol, triglycerides, and high-density and low-density cholesterol by use of routine methods at our laboratory.

STATISTICAL ANALYSES

Data analyses were performed with SPSS (version 12). All continuous variables were skewed and were therefore log-transformed to approach gaussian distribution before applying tests that propose such a distribution of the data.

One-way ANOVA was used for multiple comparisons. The post hoc Tamhane test was performed to identify the significantly different group means when ANOVA was significant. Correlations between different variables were examined by Spearman ρ test. All tests were 2-sided; P values <0.05 were considered statistically significant.

Results

The main characteristics of the study population according to disease are presented in Table 1 in the online Data Supplement. Concentrations of plasma/serum and CSF vitamin biomarkers are shown in Tables 1 and 2. Patients with polyneuropathy were significantly older than control patients and had higher ratios of CSF:serum albumin. Concentrations of blood markers (tHcy, Cys, SAH, and SAM) were higher in patients with polyneuropathy than in control patients (Table 1). Nevertheless, these results seem to be related to the older age of patients with polyneuropathy, because the differences were no longer significant after adjusting for age and CSF:serum albumin ratio (Table 1).

Patients with dementia were much older than the control patients (see Table 1 in the online Data Supplement). Comparison between CSF and blood markers was not performed, because adjustment for age was not possible in this case. Patients with multiple sclerosis had higher serum concentrations of holoTC in addition to lower median concentrations of CSF SAH and higher CSF SAM:SAH ratios. Despite the marked age differences, patients with stroke showed no significant differences in any blood or CSF marker compared with control patients (Table 1).

The age of our patients seemed to affect concentrations of the metabolites in CSF. To test this, we pooled the data from all study populations and divided our patients into 4 groups according to quartiles of age (Table 3). Higher age was associated with higher blood concentrations of tHcy, Cys, MMA, SAH, SAM, and creatinine. Lower concentrations of serum folate and holoTC were also related to advanced age. Concentrations of CSF tHcy and CSF SAH increased with age, and concentrations of CSF folate and ratios of SAM:SAH in CSF decreased with increasing age (Table 3). Concentrations of CSF MMA tended to decrease with age, and concentrations of CSF holoTC and CSF SAM did not differ significantly with age (Table 3).

To investigate the association between concentrations of CSF SAH and other vitamin biomarkers, we divided concentrations of CSF SAH into quartiles (data from all patient groups; Table 4). Higher concentrations of CSF SAH were related to higher concentrations of plasma SAH and SAM and slightly lower SAM:SAH ratios in the

Table 1. Concentrations of serum/plasma and CSF vitamin and methylation markers according to disease status.[a]

	All	Control patients	Multiple sclerosis	Stroke	Peripheral neuropathy
n	182	65	31	19	36
Plasma/serum markers					
tHcy, μmol/L	10.7 (7.4–18.0)	9.4 (7.3–16.2)	10.7 (7.6–14.4)	10.2 (7.0–17.4)	11.9 (7.0–19.1)[b,c]
Cys, nmol/L	372 (180–886)	329 (150–661)	355 (157–1146)	420 (154–874)	415 (199–1038)[b,c]
MMA, nmol/L	197 (128–394)	186 (109–300)	197 (116–366)	176 (144–510)	189 (142–340)
Total vitamin B_{12}, pmol/L	249 (166–419)	242 (158–403)	266 (181–402)	253 (162–485)	270 (183–630)
Folate, nmol/L	19.6 (9.9–41.1)	19.3 (11.4–42.2)	21.1 (9.5–41.6)	20.3 (12.6–42.0)	22.3 (9.9–43.8)
Vitamin B_6, nmol/L	36.9 (13.8–100.8)	37.9 (17.8–90.6)	10.6 (16.1–168.4)	33.3 (12.2–115.6)	42.0 (15.3–199.7)
HoloTC, pmol/L	70 (32–136)	63 (32–128)	89 (35–156)[b]	64 (32–154)	81 (44–143)
SAM, nmol/L	123 (89–218)	116 (87–171)	114 (74–236)	130 (93–206)	138 (88–228)[b,c]
SAH, nmol/L	18.1 (10.0–37.9)	16.2 (9.3–27.4)	13.5 (9.9–51.5)	18.2 (10.9–48.2)	19.6 (11.0–37.2)[b,c]
SAM:SAH ratio	7.0 (3.8–13.0)	6.8 (3.8–13.8)	8.2 (3.0–14.4)	7.7 (3.4–11.9)	6.7 (4.1–10.1)
CSF markers					
tHcy, μmol/L	0.10 (0.06–0.17)	0.09 (0.06–0.16)	0.08 (0.05–0.16)	0.08 (0.06–0.15)	0.11 (0.07–0.18)[b,c]
Cys, nmol/L	49 (22–98)	54 (17–108)	42 (22–105)	48 (18–105)	34 (18–75)
MMA, nmol/L	359 (267–552)	359 (266–574)	367 (281–525)	426 (333–600)	326 (214–511)
Folate, nmol/L	19.5 (13.9–26.6)	20.7 (14.1–27.7)	20.5 (14.6–29.5)	19.2 (14.2–26.4)	18.6 (12.9–23.5)[b,c]
HoloTC, pmol/L	16 (4–27)	16 (6–23)	16 (3–26)	17 (4–73)	17 (7–39)
SAM, nmol/L	267 (180–356)	268 (197–355)	305 (151–389)	273 (172–339)	268 (187–385)
SAH, nmol/L	13.5 (8.4–24.1)	13.2 (7.7–24.0)	10.2 (7.4–16.7)[b]	13.5 (7.1–24.8)	15.5 (9.1–22.5)[b,c]
SAM:SAH ratio	19 (10–34)	20 (11–39)	28 (13–42)[b]	22 (10–30)	18 (12–24)
P-tau$_{(181P)}$, ng/L	39 (20–79)	35 (18–63)	37 (22–68)	48 (19–107)	49 (26–101)[b]
β-Amyloid(1–42), ng/L	651 (374–1060)	739 (444–1092)	552 (340–737)[b]	587 (327–919)	606 (520–1000)

[a] Data are median (10th–90th percentile).

[b] $P<0.05$ vs control group (ANOVA and post hoc Tamhane test).

[c] Differences no longer significant after adjusting for age and CSF:serum albumin.

Table 2. CSF and blood markers in patients with dementia (n = 31).

	Median (10th–90th percentile)
Plasma/serum markers	
tHcy, μmol/L	12.9 (8.2–28.2)
Cys, nmol/L	491 (247–1764)
MMA, nmol/L	293 (149–681)
Total vitamin B_{12}, pmol/L	195 (145–364)
Folate, nmol/L	16.1 (5.4–27.1)
Vitamin B_6, nmol/L	19.5 (8.0–45.4)
HoloTC, pmol/L	58 (20–133)
SAM, nmol/L	159 (95–369)
SAH, nmol/L	25.1 (11.1–61.7)
SAM:SAH ratio	6.9 (1.6–12.3)
CSF markers	
tHcy, μmol/L	0.10 (0.06–0.32)
Cys, nmol/L	47 (25–125)
MMA, nmol/L	419 (267–746)
Folate, nmol/L	18.4 (12.3–27.4)
HoloTC, pmol/L	11 (4–23)
SAM, nmol/L	250 (180–356)
SAH, nmol/L	15.2 (9.7–30.0)
SAM:SAH ratio	17 (7–29)
P-$tau_{(181P)}$, ng/L	36 (21–95)
β-Amyloid(1–42), ng/L	666 (290–1090)

plasma. Moreover, higher CSF SAH was associated with lower concentrations of serum folate and higher tHcy in serum. Similar associations between CSF SAH and concentrations of other vitamin biomarkers in the CSF were observed. Thus, higher concentrations of CSF SAH were associated with higher CSF tHcy and Cys, lower CSF folate, and lower CSF SAM:SAH ratio.

One very important association was observed between concentrations of CSF SAH and CSF P-tau (Table 4)—a highly significant direct correlation was found (Fig. 1A). CSF P-tau correlated negatively with SAM:SAH ratio ($r = -0.40$, $P < 0.001$). Furthermore, a negative correlation was found between CSF P-tau and CSF folate (Fig. 1B). The correlations between CSF P-tau and either CSF SAH, CSF SAM:SAH, or CSF folate remained significant after adjusting for age.

Fig. 2 shows median and 25th–75th percentiles of CSF SAH according to concentration of P-tau and age (<41, 41– 60, and >60 years). In addition to the influence of age on concentrations of CSF SAH, we found among each age group that patients who had higher concentrations of P-tau also had higher concentrations of CSF SAH compared with patients of similar age and lower CSF P-tau.

We applied multiple backward regression analyses to find factors that significantly predict CSF tHcy, CSF SAH, and CSF SAM. Concentrations of CSF tHcy were predicted by CSF Cys ($\beta = 0.478$), CSF folate ($\beta = -0.403$), CSF albumin ($\beta = 0.349$), and age ($\beta = 0.298$). Significant predictors of concentrations of SAH in the CSF were CSF tHcy ($\beta = 0.377$, $P < 0.001$) and age ($\beta = 0.195$, $P = 0.002$). Concentrations of CSF SAM were not predicted by any marker estimated in this study. Moreover, concentrations of Cys correlated with SAM in the plasma ($r = 0.510$, $P < 0.001$), and this correlation was weaker in CSF ($r = 0.174$, $P = 0.041$).

Table 3. Blood and CSF markers according to quartile of age.[a]

	Quartile 1, 17–40 years	Quartile 2, 41–55 years	Quartile 3, 56–68 years	Quartile 4, 69–86 years	*P* (ANOVA)
Plasma/serum markers					
tHcy, μmol/L	9.0 (6.0–12.7)	10.2 (7.8–20.2)[b]	11.0 (7.9–15.0)[b]	14.0 (8.1–28.4)[b]	<0.001
Cys, nmol/L	246 (129–472)	388 (188–844)[b]	362 (197–877)[b]	510 (283–1950)[b]	<0.001
MMA, nmol/L	166 (90–294)	179 (133–299)	196 (147–370)[b]	253 (146–621)[b]	<0.001
Total B_{12}, pmol/L	253 (165–433)	259 (173–435)	243 (162–424)	246 (162–422)	0.699
Folate, nmol/L	18.8 (11.9–42.8)	21.9 (10.5–43.7)	21.3 (12.7–42.8)	15.2 (7.4–25.8)[b]	<0.001
HoloTC, pmol/L	70 (26–128)	71 (41–128)	73 (39–163)	57 (26–139)	0.077
SAM, nmol/L	106 (73–143)	116 (89–179)	132 (97–219)[b]	164 (105–327)[b]	<0.001
SAH, nmol/L	12.8 (8.6–27.0)	16.4 (10.4–29.2)	19.8 (11.8–37.3)[b]	25.6 (13.8–79.7)[b]	<0.001
SAM:SAH ratio	8.3 (3.4–14.1)	7.3 (4.1–12.2)	7.5 (3.8–12.7)	6.1 (2.8–11.2)	0.079
Creatinine, μmol/L	66.3 (53.0–88.4)	70.7 (53.0–97.2)	79.6 (53.0–106.1)	88.4 (53.0–189.2)[b]	<0.001
CSF markers					
tHcy, μmol/L	0.07 (0.05–0.12)	0.10 (0.06–0.17)	0.10 (0.06–0.14)	0.13 (0.09–0.24)[b]	<0.001
Cys, nmol/L	53 (17–110)	42 (19–97)	33 (18–70)	65 (27–131)	0.002
MMA, nmol/L	392 (251–776)	359 (289–509)	348 (261–522)	359 (258–554)	0.188
Folate, nmol/L	21.0 (16.3–28.9)	19.8 (12.3–27.4)	19.7 (15.3–23.1)	16.9 (11.9–21.8)[b]	<0.001
HoloTC, pmol/L	14 (5–25)	17 (3–23)	18 (6–38)	14 (4–29)	0.204
SAM, nmol/L	254 (174–332)	270 (154–375)	259 (181–356)	274 (190–374)	0.464
SAH, nmol/L	10.5 (6.5–14.4)	12.7 (7.9–20.2)	15.3 (9.1–25.4)[b]	16.7 (10.3–30.0)[b]	<0.001
SAM:SAH ratio	26 (16–40)	19 (11–36)	16 (8–31)[b]	17 (8–29)[b]	<0.001

[a] Data are median (10th–90th percentile).
[b] $P < 0.05$ according to post hoc Tamhane test vs quartile 1.

Table 4. Concentrations of plasma/serum and CSF biomarkers of B vitamins according to quartile of CSF-SAH.[a]

	Quartile 1, 4.7–10.1 nmol/L	Quartile 2, 10.2–15.4 nmol/L	Quartile 3, 15.5–17.4 nmol/L	Quartile 4, 17.5–39.3 nmol/L	*P* (ANOVA)
Age, years	41 (16)	44 (18)	57 (15)[b]	64 (13)[b]	<0.001
Plasma/serum markers					
tHcy, μmol/L	10.2 (3.1)	10.0 (8.1)	12.1 (9.6)	12.6 (5.0)[b]	0.019
Cys, nmol/L	308 (320)	388 (686)	451 (644)[b]	491 (571)[b]	0.007
MMA, nmol/L	201 (117)	188 (94)	214 (138)	262 (256)	0.018
Total vitamin B_{12}, pmol/L	279 (1159)	280 (506)	274 (1111)	254 (90)	0.851
Folate, nmol/L	22.3 (16.2)	21.4 (11.8)	17.8 (13.1)	16.5 (10.2)[b]	0.036
HoloTC, pmol/L	68 (37)	56 (40)	62 (44)	69 (42)	0.349
SAM, nmol/L	110 (34)	130 (128)	134 (62)	157 (101)[b]	0.002
SAH, nmol/L	15.2 (6.8)	17.0 (11.8)	22.2 (41.7)[b]	24.4 (39.9)[b]	0.001
SAM:SAH	7.3 (3.5)	7.7 (3.1)	6.1 (4.5)	6.4 (3.7)	0.162
Creatinine, μmol/L	69.8 (14.4)	73.3 (16.9)	97.0 (94.3)	93.9 (56.3)	0.032
CSF markers					
tHcy, μmol/L	0.08 (0.04)	0.10 (0.26)[b]	0.10 (0.03)[b]	0.14 (0.19)[b]	<0.001
Cys, nmol/L	42 (27.5)	46 (33)	46 (22)[b]	49 (35)[b]	0.666
MMA, nmol/L	414 (318)	372 (130)	363 (111)	372 (127)	0.334
Folate, nmol/L	20.8 (4.2)	20.2 (5.3)	18.4 (4.4)	17.1 (4.8)[b]	0.003
HoloTC, pmol/L	11 (12)	13 (24)	13 (7)	14 (20)	0.431
SAM, nmol/L	247 (71)	270 (58)	260 (61)	258 (72)	0.450
SAM:SAH	29.6 (9.4)	23.3 (6.1)[b]	17.1 (4.3)[b]	11.1 (3.9)[b]	<0.001
P-tau$_{(181P)}$, ng/L	29.7 (12.4)	34.5 (12.6)	44.9 (26.8)[b]	49.6 (34.4)[b]	<0.001
β-Amyloid(1–42), ng/L	606 (188)	566 (237)	630 (250)	702 (239)	0.135

[a] Data are geometric mean (SD).
[b] $P < 0.05$ vs quartile 1 (post hoc Tamhane test).

Discussion

Concentrations of tHcy or related biomarkers in CSF have been tested in only a few studies *(18, 25)*. Human studies demonstrated decreased SAM or increased tHcy or SAH in brain or CSF from patients with certain disorders of the central nervous system *(26, 27)*. The most remarkable finding in this study is that increased concentrations of P-tau in the brain may be related to disturbed Hcy metabolism or methylation status. These findings may be a plausible explanation for the association between Hcy metabolism and neurodegenerative diseases.

Previous reports documented higher serum or plasma concentrations of tHcy and MMA in patients with dementia compared with nondemented individuals *(5)*. Moreover, lower concentrations of CSF folate were observed in late-onset dementia patients compared with nondemented patients *(28)*. Our study has shown that concentrations of folate decrease and concentrations of tHcy

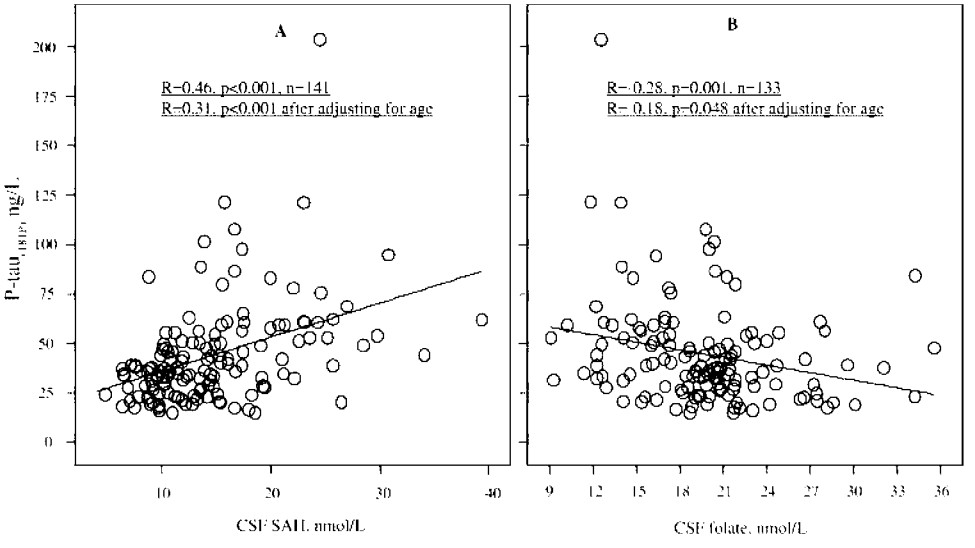

Fig. 1. Correlation between concentrations of P-tau$_{(181P)}$ and CSF SAH and CSF folate (Spearman test).

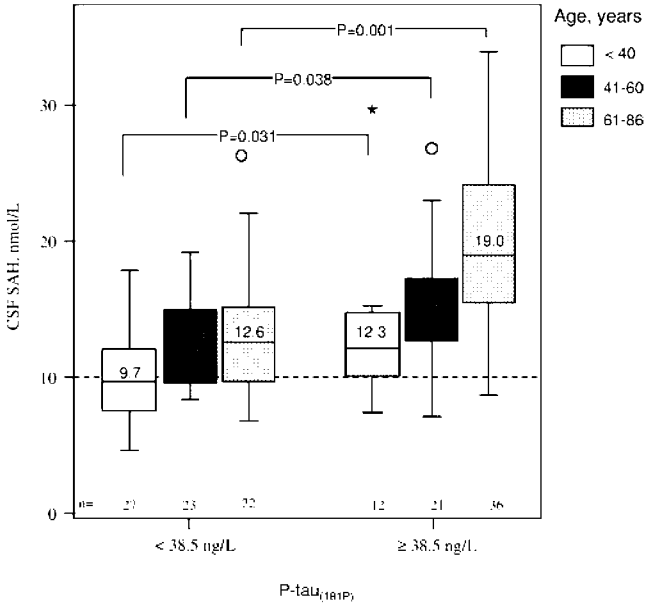

Fig. 2. Box plot showing concentrations of CSF SAH according to CSF P-tau$_{(181P)}$ and age.

Concentrations of P-tau$_{(181P)}$ were divided into 2 groups (< or ≥ median value; 38.5 ng/L) in the pooled data (P-tau data available from 141 patients). *P* values are according to the Mann–Whitney test. Median CSF SAH values are indicated in the Fig.

increase in CSF with age (Table 3). CSF folate was a stronger predictor ($\beta = -0.403$) of CSF tHcy than age ($\beta = 0.298$). The positive correlation between plasma and CSF concentrations of tHcy has been confirmed by a previous study *(29)*. Therefore, increase of tHcy in the blood may indicate its increase in the brain, where tHcy can have many neurotoxic effects.

Disturbed methyl group metabolism in the brain might be closely related to age and to methyl group metabolism in other parts of the body. As suggested by our results, increase of CSF SAH with age might be related to increased concentrations of tHcy in the plasma or in the CSF (Table 3). Hcy is converted into SAH via SAH-hydrolase. The reaction in the SAH direction is favored in case of excess tHcy. This is in line with in vivo results showing that Hcy administration led to increased brain SAH *(17)*. Because SAH is a potent inhibitor of many transmethylases, the lower ratio of SAM:SAH in the CSF indicates a hypomethylation state in the brain and may affect several important biological pathways.

It is of interest that aging was related to higher CSF concentrations of tHcy and SAH, in addition to lower folate and lower ratio of SAM:SAH in the CSF (Table 3). These metabolic changes with age may be very important factors that play a paramount role in the genesis of age-related disorders. In addition to the role of age as a significant modulator of CSF SAH, our data demonstrated that a lower folate status (in the circulation or in the CSF) is related to an increased concentration of SAH in the CSF (Table 4). It is plausible that aging is associated with a higher concentration of SAH in CSF via decrease of CSF folate.

The abnormal aggregation of P-tau into paired helical filaments takes several decades to develop and is considered one of the hallmarks of Alzheimer disease. Tau aggregation that takes place in the cytosol is toxic for the neurons. Our study showed that concentrations of P-tau in the CSF are associated with concentrations of folate and SAH in the CSF (Fig. 1). This association was independent of age and evident in each age group (Fig. 2). Previous results of experimental folate deficiency in neuronal cells support our results *(30)*. Folate deprivation in neuroblastoma cells induced a marked increase in tHcy concentration, in addition to an increase in the immunoreactivity of P-tau *(30)*, and the increment of P-tau was reversible after adding folate to the cultures *(30)*. Animal studies have shown that P-tau accumulates in cases of diet-induced (folate deficiency) or genetic (apolipoprotein E deficiency) oxidative stress *(31)*. Whereas SAM treatment partly restored oxidative stress, it did not reduce P-tau in apolipoprotein E–deficient animals *(31)*. Altogether, these results suggest that P-tau accumulates in folate deficiency and in cases of oxidative stress, but this phenotype is probably reversible, at least in short-term deficiency. Because reducing P-tau might lead to improved memory function, independent of paired helical filaments *(32)*, tHcy-lowering treatment may improve memory function via reducing P-tau. This effect should be tested in folate-deficient individuals after vitamin supplementation.

There is a plausible biological mechanism by which folate deprivation or increased SAH can cause increased P-tau. The dephosphorylation of P-tau depends on the activity of PP2A (see Fig. 1 in the online Data Supplement). Activating this phosphatase is accomplished by a specific transmethylase, the phosphatase methyltransferase 1, which is SAM dependent *(33)*. Low concentrations of SAM or a low SAM:SAH ratio results in a lower activity of PP2A and accumulation of P-tau *(20)*. This is in line with our results, in which a lower SAM:SAH ratio was negatively related to concentrations of P-tau in the CSF. Earlier studies demonstrated that the protein concentration of phosphatase methyltransferase 1 and that of the methylated C subunit of PP2A were ~40% lower in frontal and temporal extracts from Alzheimer disease patients compared with control patients *(34)*. In addition, PP2A activity and gene expression were markedly reduced in the brains of Alzheimer disease patients *(35)*. As a whole, these data strongly suggest that alterations in methyl group metabolism may contribute to the etiology of dementia by inhibiting dephosphorylation of P-tau.

In the brains of Alzheimer disease patients, the intracerebral deposition of β-amyloid (mainly 1–42) is the most important pathologic process leading to dementia. In contrast to β-amyloid(1–40), β-amyloid(1–42) is insoluble and can accumulate in the plaques. Concentrations of β-amyloid(1–42) are lower in CSF from patients with dementia compared with nondemented patients. The ac-

cumulation of β-amyloid is a long-term process that is thought to start at a young age. We found no association between β-amyloid(1–42) and methylation markers, vitamins, or tHcy in the whole group or in patients with dementia. Our patients, other than those with dementia, were not tested for cognitive performance, and we cannot exclude the possibility that some elderly people were slightly demented, which might affect concentrations of CSF β-amyloid(1–42). Available in vitro evidence has suggested that tHcy accelerates dementia by stimulating β-amyloid deposition in the brain *(36)*. In line with this, a positive association between plasma concentrations of tHcy and concentrations of β-amyloid has been documented in patients with neurodegenerative disease *(11)*. Another clinical study demonstrated that folate treatment lowered concentrations of plasma β-amyloid(1–40) *(37)*. The effect of SAM or B vitamin treatments on the insoluble β-amyloid(1–42) protein in CSF has not been tested.

SAM treatment protects the neurons from degeneration by several mechanisms *(38, 39)*. The protective effect of SAM was suppressed by simultaneous administration of SAH *(40)*. These results, in addition to those of our study, suggest that increased SAH, rather than reduced SAM, may be a more important mediator in neurodegeneration. Because we demonstrated that CSF tHcy is an important determinant of CSF SAH (regression analysis), keeping tHcy and SAH at low concentrations may be important for enhancing P-tau degradation.

In summary, Hcy metabolism in the circulation is closely related to that in the brain. This relation seems to extend beyond a simple exchange of tHcy, SAH, SAM, or the vitamins across the blood–brain barrier. Higher SAH can cause P-tau accumulation, increasing the risk for neurodegenerative diseases. The response of P-tau to B vitamin treatment in deficient individuals needs to be investigated.

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References

1. Mulder C, Schoonenboom NS, Jansen EE, Verhoeven NM, van Kamp GJ, Jakobs C, et al. The transmethylation cycle in the brain of Alzheimer patients. Neurosci Lett 2005;386:69–71.
2. Hoffman DR, Marion DW, Cornatzer WE, Duerre JA. S-Adenosylmethionine and S-adenosylhomocysteine metabolism in isolated liver. Effects of L-methionine, L-homocystein, and adenosine. J Biol Chem 1980;255:10822–7.
3. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, et al. S-Adenosylmethionine and methylation. FASEB J 1996; 10:471–80.
4. Weir DG, Scott JM. The biochemical basis of the neuropathy in cobalamin deficiency. Baillieres Clin Haematol 1995;8:479–97.
5. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. Arch Neurol 1998;55:1449–55.
6. Prudova A, Bauman Z, Braun A, Vitvitsky V, Lu SC, Banerjee R. S-Adenosylmethionine stabilizes cystathionine beta-synthase and modulates redox capacity. Proc Natl Acad Sci U S A 2006;103: 6489–94.
7. McKeever MP, Weir DG, Molloy A, Scott JM. Betaine-homocysteine methyltransferase: organ distribution in man, pig and rat and subcellular distribution in the rat. Clin Sci (Lond) 1991;81:551–6.
8. Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW, Reynolds EH. Homocysteine, folate, methylation, and monoamine metabolism in depression. J Neurol Neurosurg Psychiatry 2000;69:228–32.
9. Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, et al. Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr 2005;82:636–43.
10. Wright CB, Paik MC, Brown TR, Stabler SP, Allen RH, Sacco RL, et al. Total homocysteine is associated with white matter hyperintensity volume: the Northern Manhattan Study. Stroke 2005;36: 1207–11.
11. Irizarry MC, Gurol ME, Raju S, az-Arrastia R, Locascio JJ, Tennis M, et al. Association of homocysteine with plasma amyloid beta protein in aging and neurodegenerative disease. Neurology 2005; 65:1402–8.
12. Perry IJ. Homocysteine as a risk factor for cerebrovascular disease and stroke. In: Robinson K, ed. Homocysteine and Vascular Disease. Dordrecht, The Netherlands: Kluwer Academic Publishers, 2000:151–72.
13. Coull BM, Malinow MR, Beamer N, Sexton G, Nordt F, deGarmo P. Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. Stroke 1990;21:572–6.
14. Sachdev PS, Valenzuela M, Wang XL, Looi JC, Brodaty H. Relationship between plasma homocysteine levels and brain atrophy in healthy elderly individuals. Neurology 2002;58:1539–41.
15. Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med 2002;346:476–83.
16. Nurk E, Refsum H, Tell GS, Engedal K, Vollset SE, Ueland PM, et al. Plasma total homocysteine and memory in the elderly: the Hordaland Homocysteine study. Ann Neurol 2005;58:847–57.
17. Gharib A, Chabannes B, Sarda N, Pacheco H. In vivo elevation of mouse brain S-adenosyl-l-homocysteine after treatment with l-homocysteine. J Neurochem 1983;40:1110–2.
18. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006;5:228–34.
19. Ho PI, Collins SC, Dhitavat S, Ortiz D, Ashline D, Rogers E, et al. Homocysteine potentiates beta-amyloid neurotoxicity: role of oxidative stress. J Neurochem 2001;78:249–53.
20. Vafai SB, Stock JB. Protein phosphatase 2A methylation: a link between elevated plasma homocysteine and Alzheimer's Disease. FEBS Lett 2002;518:1–4.
21. Obeid R, Kostopoulos P, Knapp JP, Kasoha M, Becker G, Fassbender K, et al. Biomarkers of folate and vitamin B12 are related in blood and cerebrospinal fluid. Clin Chem 2007;53:326–33.
22. Gellekink H, van Oppenraaij-Emmerzaal D, van Rooij A, Struys EA, den Heijer M, Blom HJ. Stable-isotope dilution liquid chromatography-electrospray injection tandem mass spectrometry method for fast, selective measurement of S-adenosylmethionine and S-adenosylhomocysteine in plasma. Clin Chem 2005;51:1487–92.

23. Obeid R, Kuhlmann MK, Kohler H, Herrmann W. Response of homocysteine, cystathionine, and methylmalonic acid to vitamin treatment in dialysis patients. Clin Chem 2005;51:196–201.

24. Ulleland M, Eilertsen I, Quadros EV, Rothenberg SP, Fedosov SN, Sundrehagen E, et al. Direct assay for cobalamin bound to transcobalamin (holo-transcobalamin) in serum. Clin Chem 2002; 48:526–32.

25. Regland B, Abrahamsson L, Blennow K, Grenfeldt B, Gottfries CG. CSF-methionine is elevated in psychotic patients. J Neural Transm 2004;111:631–40.

26. Bottiglieri T, Godfrey P, Flynn T, Carney MW, Toone BK, Reynolds EH. Cerebrospinal fluid S-adenosylmethionine in depression and dementia: effects of treatment with parenteral and oral S-adenosylmethionine. J Neurol Neurosurg Psychiatry 1990;53:1096–8.

27. Surtees R, Hyland K. Cerebrospinal fluid concentrations of S-adenosylmethionine, methionine, and 5-methyltetrahydrofolate in a reference population: cerebrospinal fluid S-adenosylmethionine declines with age in humans. Biochem Med Metab Biol 1990;44: 192–9.

28. Serot JM, Christmann D, Dubost T, Bene MC, Faure GC. CSF-folate levels are decreased in late-onset AD patients. J Neural Transm 2001;108:93–9.

29. Selley ML, Close DR, Stern SE. The effect of increased concentrations of homocysteine on the concentration of (E)-4-hydroxy-2-nonenal in the plasma and cerebrospinal fluid of patients with Alzheimer's disease. Neurobiol Aging 2002;23:383–8.

30. Ho PI, Ashline D, Dhitavat S, Ortiz D, Collins SC, Shea TB, et al. Folate deprivation induces neurodegeneration: roles of oxidative stress and increased homocysteine. Neurobiol Dis 2003;14:32–42.

31. Chan A, Shea TB. Dietary and genetically-induced oxidative stress alter tau phosphorylation: influence of folate and apolipoprotein E deficiency. J Alzheimers Dis 2006;9:399–405.

32. Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, et al. Tau suppression in a neurodegenerative mouse model improves memory function. Science 2005;309:476–81.

33. Tolstykh T, Lee J, Vafai S, Stock JB. Carboxyl methylation regulates phosphoprotein phosphatase 2A by controlling the association of regulatory B subunits. EMBO J 2000;19:5682–91.

34. Sontag E, Hladik C, Montgomery L, Luangpirom A, Mudrak I, Ogris E, et al. Downregulation of protein phosphatase 2A carboxyl methylation and methyltransferase may contribute to Alzheimer disease pathogenesis. J Neuropathol Exp Neurol 2004;63:1080–91.

35. Vogelsberg-Ragaglia V, Schuck T, Trojanowski JQ, Lee VM. PP2A mRNA expression is quantitatively decreased in Alzheimer's disease hippocampus. Exp Neurol 2001;168:402–12.

36. Fuso A, Seminara L, Cavallaro RA, D'Anselmi F, Scarpa S. S-Adenosylmethionine/homocysteine cycle alterations modify DNA methylation status with consequent deregulation of PS1 and BACE and beta-amyloid production. Mol Cell Neurosci 2005;28: 195–204.

37. Flicker L, Martins RN, Thomas J, Psych JA, Taddei K, Vasikaran SD, et al. B-vitamins reduce plasma levels of beta amyloid. Neurobiol Aging 2006 [Epub ahead of print].

38. Rao AM, Baskaya MK, Maley ME, Kindy MS, Dempsey RJ. Beneficial effects of S-adenosyl-l-methionine on blood-brain barrier breakdown and neuronal survival after transient cerebral ischemia in gerbils. Brain Res Mol Brain Res 1997;44:134–8.

39. Villalobos MA, De La Cruz JP, Cuerda MA, Ortiz P, Smith-Agreda JM, Sanchez DLC. Effect of S-adenosyl-l-methionine on rat brain oxidative stress damage in a combined model of permanent focal ischemia and global ischemia-reperfusion. Brain Res 2000;883: 31–40.

40. Sato H, Hariyama H, Moriguchi K. S-Adenosyl-l-methionine protects the hippocampal CA1 neurons from the ischemic neuronal death in rat. Biochem Biophys Res Commun 1988;150:491–6.