Homocysteine metabolism is associated with cerebrospinal fluid phosphorylated tau181, but not with amyloid ß1-42 in aging and Alzheimer's Disease

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Abstract

Background: Disturbed homocysteine metabolism has been described as a risk factor for Alzheimer's Disease (AD), and may contribute to the disease pathophysiology by increasing both amyloid beta (Aß) production and phosphorylated tau (P-tau) accumulation.

Methods: In the present study we evaluated the relationship between the cerebrospinal fluid concentrations of homocysteine (Hcys), S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and 5-methyltetrahydrofolate (5-MTHF), and the markers for AD pathology Aß1-42 and P-tau181 in 98 subjects without cognitive impairment aged 16-81 years, and in 54 AD patients.

Results: In multivariate regression tests including age, gender, creatinine, and presence of the APOE ε4 allele, P-tau181 was associated with SAH (ß=0.490; $p<0.001$), 5-MTHF (ß=-0.273; $p=0.010$) levels, and SAM/SAH ratio (ß=-0.319; $p=0.013$ in controls, and with SAH (ß=0.529; $p=0.001$) in AD. In the controls aged ≥50 years (n=49), P-tau181 was associated with SAH (ß=0.394; $p=0.023$) and Hcys levels (ß=0.394; $p=0.005$), whereas in the younger controls P-tau181 was associated with SAH (ß=0.441; $p=0.004$) and the SAM/SAH ratio (ß=0.477; $p=0.003$). The levels of Aß1-42 were not associated with the CSF concentrations of Hcys, SAM, SAH or 5-MTHF neither in the AD nor in the control group.

Conclusion: The results suggest that alteration of the homocysteine metabolism is related to increased accumulation of phosphorylated tau, and may contribute to the neurofibrillary pathology in AD. This association may become relevant for the pathophysiological changes in AD decades before the clinical onset of the disease, and may explain in part the finding of altered homocysteine metabolism as a risk factor of AD.

Introduction:

Sporadic Alzheimer disease (AD) is the most common cause of dementia in the elderly. Neuropathological changes as the progressive deposition of amyloid ß protein (Aß) and the formation of neurofibrillary tangles consisting primarily of phosphorylated tau protein (P-tau) begin many years before the clinical manifestation of the disease. Related alterations of cerebrospinal fluid (CSF) Aß1-42 and P-tau concentrations can be detected already in asymptomatic older subjects at risk for AD (Sunderland, 2004), and predict cognitive decline in cognitively healthy older adults (Stomrud, 2007; Fagan, 2007) and in subjects with mild cognitive impairment (Hansson, 2006).

Disturbed homocysteine/methionine metabolism as indicated by increased plasma homocysteine, is a common finding in the elderly (Donini, 2007), and it is associated with cognitive impairment and cognitive decline over time (Riggs KM, 1996; Lehmann M 1999; Morris MS, 2001; Ravaglia 2003; Wright CB, 2004). Several epidemiologic studies have found hyperhomocysteinemia to be a strong and independent risk factor for the development of AD dementia (Seshadri et al., 2002; Blasko et al, 2006). However, the pathophysiological mechanisms linking the homocysteine metabolisms and AD are not fully understood.

In the folate and vitamin B12 dependent homocysteine metabolism, methionine is activated to S-adenosylmethionine (SAM), which serves as ubiquitous methyl-group donor and is necessary e.g. for the synthesis of neurotransmitters, neuronal membrane stability and DNA methylation (Mudd et al., 2001; Surtees et al., 1991). The demethylated product of SAM is S-adenosylhomocysteine (SAH), which can be hydrolyzed to the neurotoxic amino acid homocysteine. As these reactions are reversible, increased homocysteine levels can lead to the accumulation of SAH, which inhibits the transmethylation events involving SAM. Due to the

central role of SAM as methyl group donor, and SAH as a strong inhibitor of transmethylation reactions, lower levels of SAM and higher levels of SAH are supposed to result in a reduced methylation capacity, which is represented by the SAM/SAH ratio (Finkelstein, 1998). Homocysteine can be excreted via the urine, or degraded to cystathionine, and, further, to cysteine. Alternatively, homocysteine can be remethylated to methionine by transfer of a methyl group from the folate derivate 5-methyltetrahydrofolate (5-MTHF).

There is growing evidence from cell culture experiments and mouse models that homocysteine metabolism alterations may contribute to the AD pathology by increasing both Aß production and tau phosphorylation (Sontag, 2007; Zhang, 2007; Chan 2008; Sontag, 2008).

However, only a few studies have investigated changes of the homocysteine metabolism in the central nervous system of patients with AD. Cerebrospinal fluid (CSF) studies on the association of the homocysteine metabolism with AD revealed conflicting results (Bottiglieri, 1990; Selly, 2002, Serot, 2005) In a study on CSF levels of SAM, 5-MTHF and SAH, the authors found no differences between these metabolites in AD and controls (Mulder, 2005), whereas in another study significantly lower CSF levels of SAM were found in patients with AD compared to controls (Bottiglieri, 1990). In line with the finding of reduced concentrations of SAM in the CSF, decreased SAM levels were observed in the brains of AD patients (Morisson et al., 1996). Furthermore, a positive correlation was found between Aß and homocysteine in plasma in a study in AD and non-AD patients (Irizarry 2005), whereas a CSF study reported significant correlations of SAH and folate concentrations with P-tau181 levels in participants with different neurological diseases (Obeid et al. 2007).

In the present study we evaluated the relationship between different elements of the homocysteine metabolism and CSF markers of AD pathology. We assessed the CSF

concentrations of Hcys, SAM, SAH and 5-MTHF as well as the levels of Aß1-42 and P-tau181 in adults without cognitive impairment over the life span and in AD patients. Additionally, we determined the apolipoprotein E (APOE) genotype of the study participants.

Methods:

Patients

The 54 study participants with AD were referred to the Memory Clinic, Department of Psychiatry, University of Bonn, for investigation of their cognitive complaints. They met clinical diagnostic criteria for probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and Related Disorders Association (McKhann, 1984) and DSM-IV criteria for dementia of the Alzheimer type.

The AD diagnosis was based on neuropsychological and clinical evaluation and was made by a consensus conference of psychiatrists and neuropsychologists prior to the metabolites measurements.

The presence of relevant vascular cerebral damage was excluded for all study participants with AD by computed tomography or magnet resonance tomography and the Hachinsky Ischemic Score (score<4) (Wade and Hachinsky, 1986)

As control subjects we recruited 98 consecutive patients (49 subjects aged ≥50 years and 49 subjects aged <50 years) who underwent lumbar puncture at the Department of Neurology, University of Bonn, for different indications such as exclusion of CNS inflammation, exclusion of aneurysmal subarachnoid hemorrhage or exclusion of meningitis.

Exclusion criteria included history or clinical evidence of cognitive decline, Mini-Mental State Examination scores≤26 (Folstein, 1975), regular intake of vitamin supplements,

inflammatory diseases of the central nervous system, and other severe or unstable illness such symptomatic cardiac disease, renal or hepatic dysfunction, insulin-dependent diabetes mellitus, untreated thyroidal dysfunction, or excessive alcohol intake. In addition, patients with CSF samples indicating blood brain barrier disturbances or inflammatory signs, defined as CSF whole protein content >500mg/dl and more than 5 leucocytes/mm^3, were excluded from both the AD group and controls. The study was approved by the local ethics committee. Written informed consent was obtained from all study participants or their legal representatives.

Table 1 lists detailed information about the groups.

CSF collection

Diagnostic lumbar punctures were performed at the Departments of Neurology or Psychiatry, University of Bonn. A standardized technique with a 20G “atraumatical” spinal needle and a sitting or lying position for the patient was applied. CSF samples were immediately put on dry ice and then stored at -80 °C until assay procedures.

Biochemical measurements and APOE genotyping:

CSF was analyzed by tandem mass spectrometry for 5-MTHF, SAM, SAH and homocysteine as described previously (Struys, 2000; Smith, 2006. Homocysteine was measured in CSF by HPLC using fluorescence detection as previously described (Ubbink, 1991). Serum creatinine was measured during the clinical routine. Leukocyte genomic DNA was isolated with the Qiagen blood isolation kit (Qiagen, Hilden, Germany). The APOE genotype was determined as previously described (Hixson, 1991).

Kommentar [P1]: Textbaustein Piotr

Statistical analysis:

All biomarker data were normally distributed according to the Kolmogorov-Smirnov test. Within groups, multivariate backward regression tests with P-tau181 and Aß1-42, respectively, as the dependent variables were performed to investigate for associations with the Hcys, SAM, SAH and 5-MTHF, and entering age, gender, creatinine, and presence or absence of APOE ε4 allele as further variables. The associations between P-tau181 and the SAM/SAH ratio, and between Aß1-42 and the SAM/SAH ratio were separately analyzed in backward regression models including age, gender, creatinine, and presence or absence of APOE ε4 allele. Additionally, Aß1-42 was included in all regression models with P-tau181 as the dependent variable, and P-tau181 in the models with Aß1-42 as the dependent variable. In a further step, the younger control subjects (age<50 years; n=49) were analyzed separately to investigate whether associations between the parameters of the homocysteine metabolism and CSF markers for AD can be observed in this subgroup or may be related only to older age. All statistical analyses were performed using the statistical analysis software package SPSS 14.0 for Windows.

Results:

P-tau181 was associated with SAH (T=5.074; ß=0.490; p<0.001), 5-MTHF (T=-2.701; ß=-0.273; p=0.010), gender (T=2.097; ß=0.227; p=0.042) and Aß1-42 levels (T=3.457; ß=0.345; p=0.001) in the control group (model: F_{1-45}=15.494, p<0.001). Within the AD group (model: F_{1-27}=6.056, p=0.001), SAH (T=3.685; ß=0.529; p=0.001) and Aß1-42 levels (T=-2.268; ß=-0.319; p=0.031). The CSF SAM/SAH ratio was associated with P-tau181 levels (model: F_{1-46}=13.311, p<0.001; T=-2.575; ß=-0.319; p=0.013) only in the control group.

When analyses were separately performed in the subgroup of younger controls, in the final model (F_{1-23}=8.855, p<0.001) P-tau181 was associated with SAH (T=3.166; ß=0.441; p=0.004) and Aß1-42 levels (T=2.447; ß=0.339; p=0.022). In the control subjects aged ≥50

years (model: F_{1-18}=6.918, p=0.002), P-tau181 was associated with SAH (T=2.475; ß=0.394; p=0.023) and Hcys levels (T=3.217; ß=0.394; p=0.005). Furthermore, P-tau181 was associated with the SAM/SAH ratio in the younger (F_{1-28}=11.344, p<0.001; T=-3.290; ß=0.477; p=0.003) and the older subgroups.

The levels of Aß 1-42 were not significantly associated with the CSF concentrations of Hcys, SAM, SAH, 5-MTHF or the SAM/SAH ratio neither in the AD nor in the control group (not shown).

Discussion:

Despite substantial evidence from epidemiological studies that disturbed homocysteine metabolism increases the risk and contribute to the clinical course of AD, possible underlying pathophysiological mechanisms are not entirely explained. In the present study we found CSF metabolites of the homocysteine cycle to be associated with CSF P-tau181 levels, which suggest that alterations of the homocysteine metabolism in the central nervous system may contribute to increased tau phosphorylation, a hallmark of AD pathology.

In both the AD and the control group we found strong associations of P-tau181 levels with SAH concentrations in the CSF. In addition, P-tau181 levels were associated with 5-MTHF CSF concentrations and the SAM/SAH ratio in the control group, and with Hcys levels in the subgroup of older control participants. Several studies have shown that alterations of the homocysteine metabolism may result in increased phosphorylation of the tau protein. The insufficient methylation of protein phosphatase 2A (PP2A), which depends on SAM as methyl group donor, and is necessary for efficient tau dephosphorylation was proposed as a possible mechanism leading to the accumulation of P-tau in AD (Vafai and Stock, 2002).

As demonstrated in a study in neuroblastoma cells, incubation with SAH leads to reduced methylation of PP2A, which is associated with dose dependent enhanced tau phosphorylation and accumulation of P-tau (Sontag, 2007). Other studies showed that folate deprivation increases tau phosphorylation by homocysteine induced calcium influx and by inhibition of phosphatase activity in cultured cells, and enhances the brain levels of P-tau in mice (Chan, 2008; Sontag, 2008). Furthermore, experimental in vivo homocysteine administration leads to increased brain SAH (Gharib 1983), and induces AD-like tau hyperphosphorylation in rat hippocampus by inactivating PP2A (Zhang, 2007).

In humans, post mortem studies revealed reduced hippocampal PP2A mRNA expression (Vogelsberg-Ragaglia, 2001) and decreased PP2A methylation levels in the brains of patients with AD compared to controls (Sontag, 2004). In addition, the cerebral loss of PPMT (a specific metyltransferase which catalyze the PP2A methylation) closely paralleled the severity of tau pathology, but not the amyloid plaque burden (Sontag, 2004). In a previous CSF study in participants with different neurological diseases including 9 AD patients the authors reported significant correlations of SAH and folate levels with CSF P-tau181 (Obeid, 2007). Together with these studies, our findings strongly suggest that alterations of the homocysteine metabolism contribute to tau hyperphosphorylation, which is reflected by increased CSF concentrations of P-tau181. Furthermore, we observed this association not only in AD, but also in the cognitively healthy controls, and in particular in the subgroup of younger controls which suggests that homocysteine metabolism disturbance may become relevant for tau hyperphosphorylation decades before the first clinical signs of AD. This possibly contributes to the pathophysiological changes at pre-clinical disease stages, and may explain in part the finding of altered homocysteine metabolism as a risk factor of AD.

Another central feature of the AD pathology is the cerebral aggregation and deposition of Aß1-42 in the form of amyloid plaques, which is paralleled by decreased CSF Aß1-42

concentrations. In experimental studies, disturbed homocysteine metabolism has been linked to different possible mechanisms resulting in overproduction of Aß1-42. For example, reduced PP2A methylation after cell incubation with SAH was found to be associated with increased secretion of ß-secretase cleaved amyloid precursor protein fragments and Aß peptides (Sontag et al., 2004). Other studies have shown that homocysteine metabolism alterations i.e. resulting from B-vitamin and folate deficiency modify DNA methylation status with consequent enhance of presenilin1 and ß-secretase expression, which leads to increased Aß production in cell cultures (Scarpa, 2003; Fuso, 2005) and in mice (Fuso, 2008; Chan and Shea, 2007).

In line with these findings, positive correlations between plasma homocysteine and plasma Aß levels have been reported in a sample of patients with AD, mild cognitive impairment, Parkinson's Disease and healthy controls (Irizarry 2005). Furthermore, B-vitamins administration reduced the plasma levels of Aß in a study in older men (Flicker 2008). In a previous CSF study in participants with different neurological diseases, however, no association was found between homocysteine metabolism parameters and Aß1-42 levels (Obeid, 2007). In accordance with this later report, in our study the CSF levels of Aß 1-42 did not correlate with the CSF concentrations of Hcys, SAM, SAH and 5-MTHF, or the SAM/SAH ratio neither in the AD nor in the control group. These results do not support the hypothesis that disturbed homocysteine metabolism substantially contributes to increased cerebral Aß production. Possible explanations for the discrepancy between these findings in the CSF and the results of experimental and plasma studies may be that other mechanisms modifying the cerebral Aß production and clearance may have more important effects on the Aß1-42 CSF levels, and that plasma Aß levels does not accurately reflect CSF Aß concentrations (Mehta, 2001).

The presence of the APOEε4 allele is a major risk factor for AD, and the APOE genotype was proposed to modify the links between disturbed homocysteine metabolism and neurodegenerative processes (Tchantchou, 2006; Chan and Shea, 2007). Some community-based studies (Elias, 2008), but not all (Dufouil, 2003) have found that the presence of the APOEε4 allele influences the association between plasma homocysteine and cognitive performance in cognitively healthy subjects. Experimental studies demonstrated that baseline levels of brain SAM were lower in APOE knockout mice in comparison to control mice. Furthermore, alteration of the homocysteine metabolism induced by folate deficient diet was more pronounced, and associated with increased gamma-secretase activity, and higher Aß levels in the APOE knockout mice (Tchantchou, 2006; Chan and Shea, 2007). However, in a study including patients with AD, mild cognitive impairment, Parkinson's Disease and healthy controls the presence of the APOEε4 allele did not influenced the association of plasma homocysteine with plasma Aß42 levels (Irizarry, 2005). In accordance with this report, in our study the presence or absence of the APOEε4 allele did not modified the associations of the metabolites with the CSF levels of Aß1-42 and P-tau181 in any group, which did not support the hypothesis that the presence of the APOEε4 allele may link the homocysteine metabolism alteration to neurodegeneration.

References

1. Finkelstein JD. The metabolism of homocysteine: pathways and regulation. Eur.J.Pediatr. 1998;157 Suppl 2:S40-S44.

2. 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;23:383-388.

3. Serot JM, Barbe F, Arning E, et al. Homocysteine and methylmalonic acid concentrations in cerebrospinal fluid: relation with age and Alzheimer's disease. J Neurol Neurosurg Psychiatry 2005;76:1585-1587.

4. Mulder C, Schoonenboom NS, Jansen EE, et al. The transmethylation cycle in the brain of Alzheimer patients. Neurosci Lett 2005;386:69-71.

5. 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;53:1096-1098.

6. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-944.

7. Wade J, Hachinski V. Revised ischemic score for diagnosing multi-infarct dementia. J Clin Psychiatry 1986;47:437-438.

8. Smith DE, Kok RM, Teerlink T, Jakobs C, Smulders YM. Quantitative determination of erythrocyte folate vitamer distribution by liquid chromatography-tandem mass spectrometry. Clin Chem Lab Med 2006;44:450-459.

9. Struys EA, Jansen EE, de Meer K, Jakobs C. Determination of S-adenosylmethionine and S-adenosylhomocysteine in plasma and cerebrospinal fluid by stable-isotope dilution tandem mass spectrometry. Clin Chem 2000;46:1650-1656.

10. Ubbink JB, Hayward Vermaak WJ, Bissbort S. Rapid high-performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr 1991;565:441-446.

11. Hixson JE, Vernier DT, Powers PK. Detection of SstI restriction site polymorphism in human APOC3 by the polymerase chain reaction. Nucleic Acids Res 1991;19:196.

12. Elias MF, Robbins MA, Budge MM, et al. Homocysteine and cognitive performance: modification by the ApoE genotype. Neurosci Lett 2008;430:64-69.

13 Sontag JM, Nunbhakdi-Craig V, Montgomery L, et al. Folate deficiency induces in vitro and mouse brain region-specific downregulation of leucine carboxyl methyltransferase-1 and protein phosphatase 2A B(alpha) subunit expression that correlate with enhanced tau phosphorylation. J Neurosci. 2008 Nov 5;28(45):11477-87.

Blasko I, Jellinger K, Kemmler G, Krampla W, Jungwirth S, Wichart I, Tragl KH, Fischer P. Conversion from cognitive health to mild cognitive impairment and Alzheimer's disease: prediction by plasma amyloid beta 42, medial temporal lobe atrophy and homocysteine. Neurobiol Aging 2008;29(1):1-11.

Bottiglieri T, Godfrey P, Flynn T, Carney MW, Toone BK, Reynolds EH. Cerebrospinal fluid S-adenosylmethionine in depression and dementia: effects of

Formatiert: Englisch Großbritannien

treatment with parenteral and oral S-adenosylmethionine. J Neurol Neurosurg Psychiatry 1990;53(12):1096-8.

Chan A, Shea TB. Folate deprivation increases presenilin expression, gamma-secretase activity, and Abeta levels in murine brain: potentiation by ApoE deficiency and alleviation by dietary S-adenosyl methionine. J Neurochem 2007;102(3):753-60.

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(11):1449-55.

Donini LM, De Felice MR, Cannella C. Nutritional status determinants and cognition in the elderly. Arch Gerontol Geriatr 2007;44 Suppl 1:143-53.

Elias MF, Robbins MA, Budge MM, Elias PK, Dore GA, Brennan SL, Johnston C, Nagy Z. Homocysteine and cognitive performance: modification by the ApoE genotype. Neurosci Lett 2008;430(1):64-9.

Finkelstein JD. The metabolism of homocysteine: pathways and regulation. EurJPediatr 1998;157 Suppl 2:S40-S4.

Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12(3):189-98.

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(1):195-204.

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(3):228-34.

Hixson JE, Vernier DT, Powers PK. Detection of SstI restriction site polymorphism in human APOC3 by the polymerase chain reaction. Nucleic Acids Res 1991;19(1):196.

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34(7):939-44.

Morris MS, Jacques PF, Rosenberg IH, Selhub J. Hyperhomocysteinemia associated with poor recall in the third National Health and Nutrition Examination Survey. Am J Clin Nutr 2001;73(5):927-33.

Morrison LD, Smith DD, Kish SJ. Brain S-adenosylmethionine levels are severely decreased in Alzheimer's disease. JNeurochem 1996;67(3):1328-31.

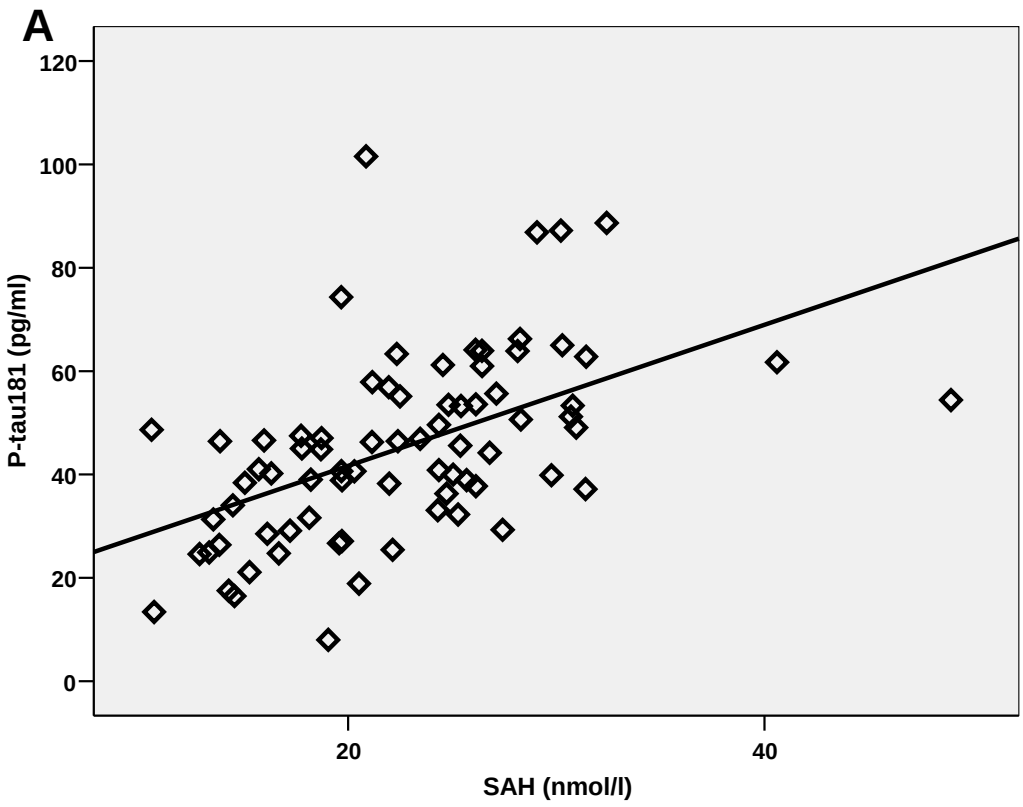
Mudd SH, Levy HL, Kraus JP. Disorders of transsulfuration. Vol. The Metabolic and Molecular Bases of Inherited Disease. New York: Mc Graw-Hill: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Kinzler K, Vogelstein B. Editors; 2001. p 2007-56.

Table 1 : Subject characteristics

	Controls (n=98)	**AD (n=54)**
Age (years): mean (SD), range	50.05 (16.84), 16-81	73.04 (7.69), 56-92
Gender (m/w): n	54/44	14/40
APOEε4 (non-carrier/ carrier): n	79/17	17/37
MMSE: mean (SD), range	29.71 (0.55), 28-30	21.33 (4.27), 6-29
Cr (mg/dl): mean (SD), range	0.86 (0.17), 0.58-1.43	0.84(0.17), 0.62-1.31

Hcys (nmol/l): mean (SD), range	72.13 (44.50), 12.60-303.30	71.89 (43.55), 18.60-226.00
5-MTHF (nmol/l): mean (SD), range	41.49 (13.66), 12.10-74.30	37.65 (10.42), 16.50-64.70
SAH (nmol/l): mean (SD), range	22.04 (6.70), 10.60-49.00	26.86 (6.18), 12.60-41.60
SAM (nmol/l): mean (SD), range	204.90 (40.53), 80.00-390.70	192.63 (31.19), 123.30-264.60
SAM/SAH: mean (SD), range	10.05 (3.36), 3.39-19.97	7.61 (2.39), 3.52-14.62
P-tau181 (pg/ml): mean (SD), range	45.18 (17.82), 8.00-101.57	81.62 (33.55), 29.40-182.10
Aβ1-42 (pg/ml): mean (SD), range	930.45 (245.43), 327.00-1412.00	420.07 (158.46), 123.30-264.60

SD : Standard-Deviation; MMSE : Mini-Mental State Examination; Cr : Creatinine; Hcys : Homocysteine; 5-MTHF : 5-methyltetrahydrofolate; SAH : S-adenosylhomocysteine; SAM : S-adenosylmethionine.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P-tau181 (pg/ml)
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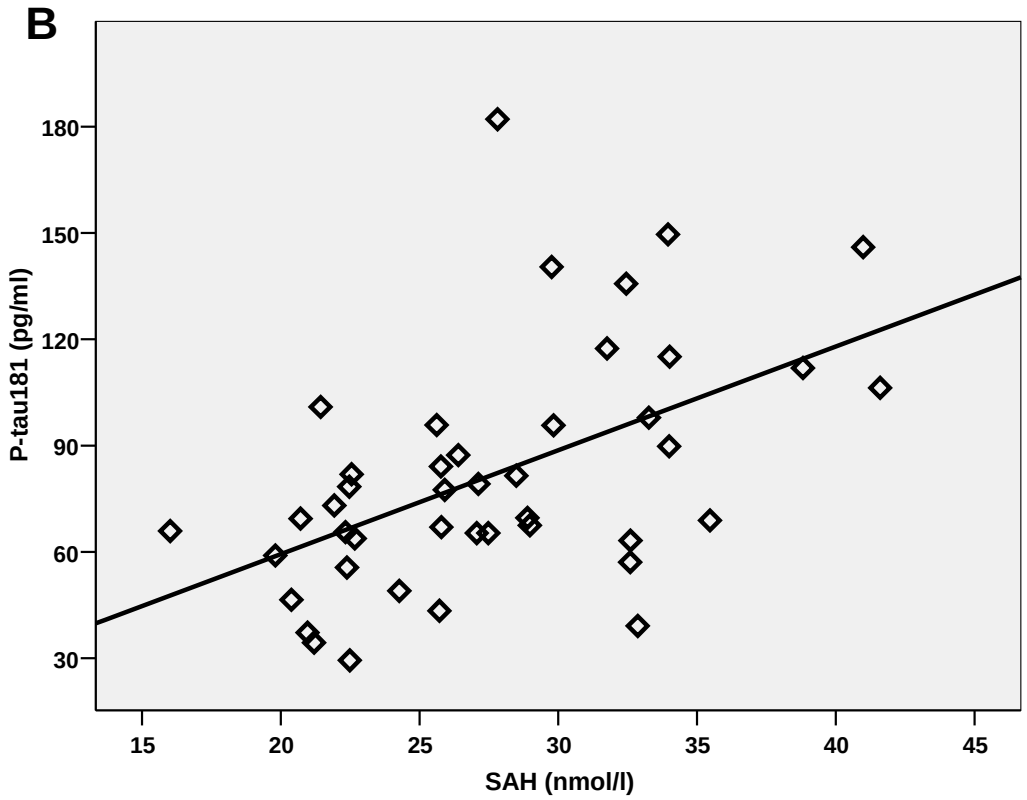
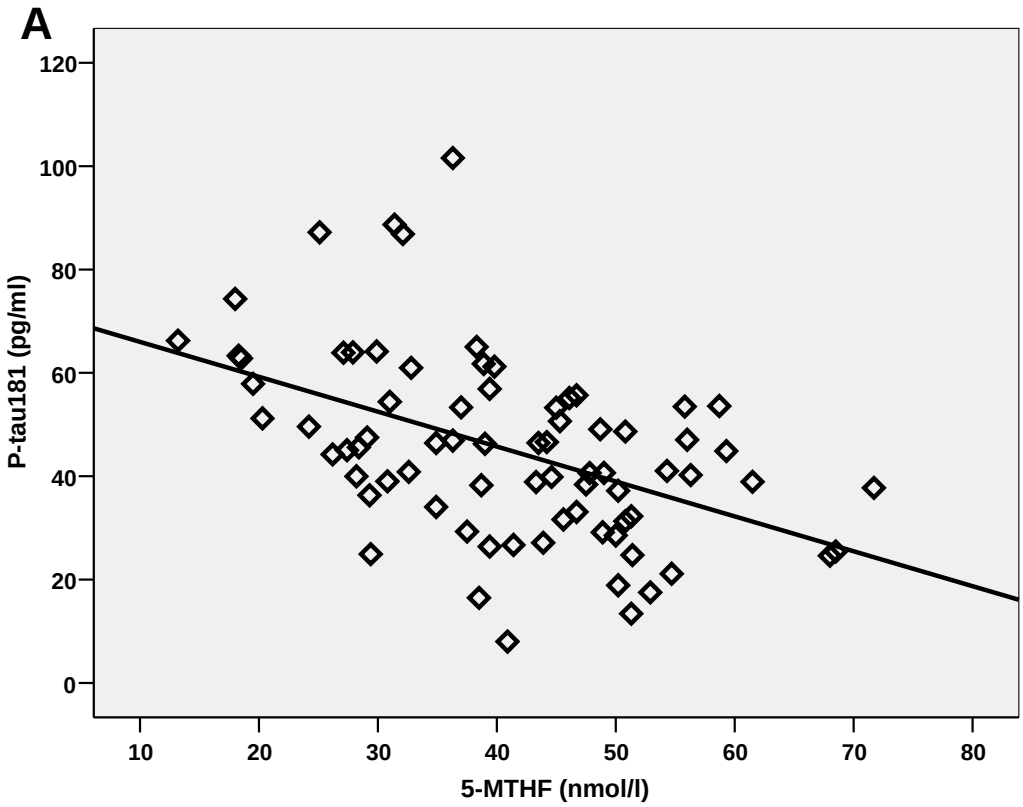


Fig. 1: Correlations between the concentrations of CSF P-tau181 and CSF SAH in (A) the control and (B) the AD group.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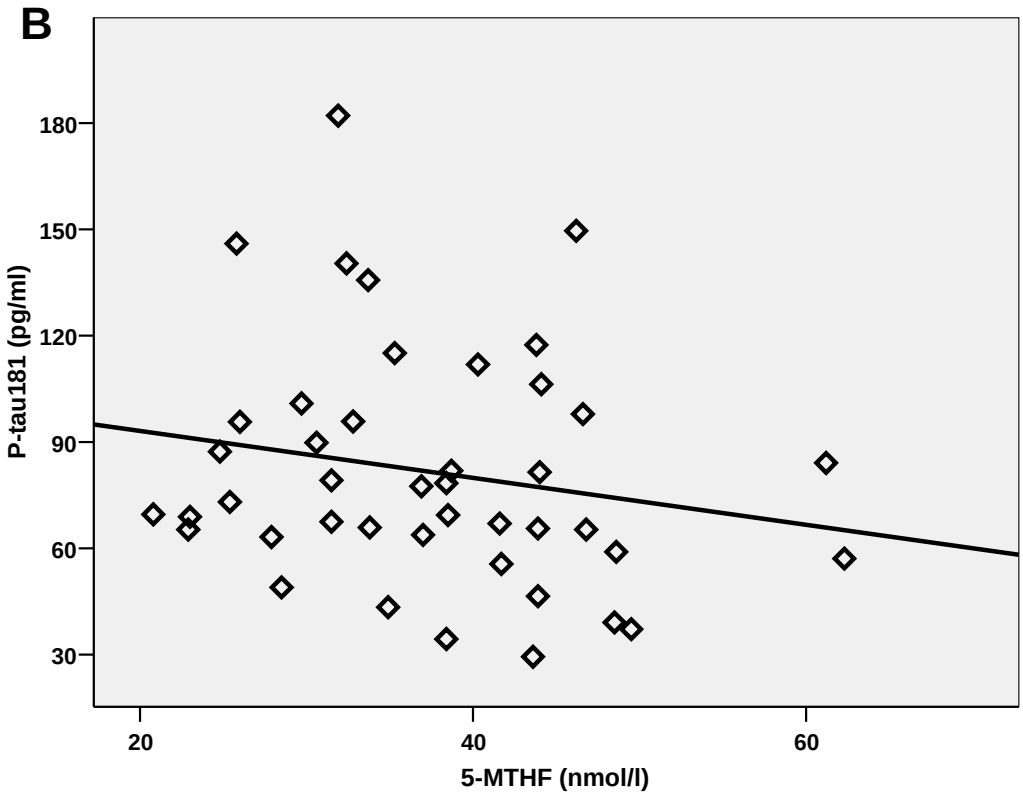

Fig. 2: Correlations between the concentrations of CSF P-tau181 and CSF 5-MTHF in (A) the control and (B) the AD group.