Association of homocysteine with plasma amyloid β protein in aging and neurodegenerative disease

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Abstract—Background: Elevated plasma total homocysteine (tHcy) is a risk factor for cardiovascular disease and is reported to be an independent risk factor for Alzheimer disease (AD) and cognitive decline. tHcy may potentiate neurotoxic and vasculopathic processes, including amyloid β protein (Aβ) metabolism, implicated in neurodegenerative diseases. Objective: To examine the relationship of plasma total tHcy levels with clinical, demographic, biochemical, and genetic factors in aging, mild cognitive impairment (MCI), AD, cerebral amyloid angiopathy (CAA), and Parkinson disease (PD). Methods: Plasma tHcy, folate, vitamin B12, creatinine, and Aβ levels were assessed in individuals evaluated in the Memory, Stroke, and Movement Disorders Units of Massachusetts General Hospital with diagnoses of AD (n = 145), MCI (n = 47), PD (n = 93), CAA (67), hypertensive intracerebral hemorrhage (hICH) (n = 25), and no dementia (n = 88). Results: The tHcy levels did not differ across AD, MCI, CAA, hICH, and nondemented control subjects but were increased in the PD group (p < 0.01). The elevated levels within the PD group were due to high tHcy in individuals taking levodopa (p < 0.0001). Increasing tHcy was associated with worse cognition in the PD cases, but not the other diagnostic groups. tHcy levels positively correlated with plasma Aβ levels even after adjustments for age and creatinine (p < 0.0001). Conclusions: Mean tHcy levels increased with age but did not discriminate diagnostic groups aside from significant elevation in patients with PD taking levodopa. The positive association between tHcy and plasma Aβ levels raises the possibility that these circulating factors could interact to affect AD risk and cognition in PD.

Homocysteine, an endogenous product of methionine metabolism, may have toxic effects on both blood vessels and neurons.1-4 Some,5-11 but not all,12-14 epidemiologic studies suggest that elevated plasma total homocysteine (tHcy) is a risk factor for dementia analyzed either in cross-sectional or prospective cohorts. Elevated tHcy is also associated with levodopa use in PD.15-21 Notably, many of the dementia studies were performed before supplementation of dietary folate in 1998.22

Plasma amyloid-β protein (Aβ) has been examined as a potential risk factor for Alzheimer disease (AD) and the related process of cerebral amyloid angiopathy (CAA) but was not consistently elevated in these conditions.23,24 Recent data from the population-based Rotterdam study, however, demonstrated an association between plasma Aβ and microvascular disease in the brain in APOE ε4 carriers,25 suggesting that Aβ might be a cause or marker of cerebrovascular dysfunction.

We tested the hypothesis that tHcy levels were elevated in mild cognitive impairment (MCI), AD, Parkinson disease (PD), or CAA relative to normal healthy volunteers in an outpatient clinic population. Because of the possibility that tHcy and Aβ might have additive or synergistic toxic effects, we also investigated associations between disease state, circulating Aβ, and circulating tHcy. We found, unexpectedly, that tHcy independently correlated with plasma Aβ40 and Aβ42 levels. This observation raises the possibility that these factors interact to potentiate neurodegeneration.

Methods. Patients. Plasma samples were obtained from patients and caregivers evaluated in the Memory and Movement Disorders Units and Stroke Service of Massachusetts General Hospital (MGH), after informed consent as part of a biomarkers study approved by the MGH Institutional Review Board.23,26 Participants had a diagnosis of AD by National Institute of Neurological and Communication Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association criteria,27 MCI by Petersen criteria,26 PD without dementia,29 CAA by Boston criteria,30 hypertensive intracerebral hemorrhage (hICH), and no dementia. The following data were collected at the time of blood collection:
Table Demographics and results

<table>
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<tr>
<th>Demographic data</th>
<th>Control</th>
<th>MCI</th>
<th>AD</th>
<th>CAA</th>
<th>hICH</th>
<th>PD</th>
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<td>47</td>
<td>145</td>
<td>67</td>
<td>25</td>
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<td>Age, y ± SD</td>
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<td>74.8 ± 7.2</td>
<td>75.9 ± 8.7</td>
<td>73.3 ± 7.5</td>
<td>67.9 ± 10.8</td>
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<tr>
<td>% Male</td>
<td>43.1</td>
<td>46.8</td>
<td>45.1</td>
<td>52.2</td>
<td>56</td>
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<tr>
<td>Duration of illness, y ± SD</td>
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<td>5.9 ± 3.1</td>
<td>N/A1</td>
<td>N/A1</td>
<td>8.5 ± 5.8</td>
</tr>
</tbody>
</table>

Biochemical data
- tHcy, μmol/L ± SD: 8.7 ± 3.2, 8.5 ± 2.4, 8.9 ± 3.2, 8.2 ± 3.4, 8.7 ± 2.4, 10.4 ± 4.4
- Folate, nmol/L ± SD: 35.2 ± 32.9, 37.4 ± 21.2, 29.9 ± 21.3, 36.6 ± 26.0, 39.5 ± 28.6, 26.0 ± 15.7
- B12, pmol/L ± SD: 390 ± 179, 435 ± 184, 429 ± 220, 416 ± 166, 362 ± 134, 360 ± 196
- Creatinine, mg/dL ± SD: 0.91 ± 0.20, 0.95 ± 0.25, 0.97 ± 0.25, 0.95 ± 0.27, 1.06 ± 0.31, 0.95 ± 0.23

Genetic data
- APOE ε4 allele frequency: 0.10, 0.34, 0.41, 0.30, 0.20, 0.11

Statistics performed on log-transformed biochemical values adjusted for age:
- * Analysis of covariance for diagnosis \( p < 0.0001; p < 0.0012 \) relative to other diagnoses except \( p = 0.08 \) relative to hypertensive intracerebral hemorrhage.
- † Analysis of covariance for diagnosis \( p = 0.02; p = 0.01 \) relative to mild cognitive impairment, cerebral amyloid angiopathy, hypertensive intracerebral hemorrhage; \( p = 0.05 \) relative to controls.
- § Analysis of covariance for diagnosis \( p = 0.03; p < 0.006 \) relative to Alzheimer disease, mild cognitive impairment, and cerebral amyloid angiopathy.
- ¶ Analysis of covariance for diagnosis \( p < 0.04; p < 0.06 \) relative to other diagnoses.
- ‡ \( p < 0.05 \) relative to controls.

MCI = mild cognitive impairment; AD = Alzheimer disease; CAA = cerebral amyloid angiopathy; hICH = hypertensive intracerebral hemorrhage; PD = Parkinson disease; N/A = not applicable (plasma collected within 2 months of diagnosis for CAA, hICH). tHcy = total homocysteine.

Results. Demographics and summary data. Homocysteine, folate, and vitamin B12 determinations were obtained in 465 outpatients with a diagnosis of AD (n = 145), MCI (n = 47), PD (n = 93), CAA (n = 67), hypertensive ICH (n = 25), and nondemented control (n = 88) (table). As previously published, the patients with AD were older, had fewer years of education, and had overrepresentation of the APOE ε4 allele relative to the control group. The MCI and CAA cases also had a greater APOE ε4 allele frequency, and the PD group had a higher proportion of men.

Clinical associations. Clinical correlations of tHcy, folate, vitamin B12, and Cr. Since elevated tHcy has been reported in AD and PD, we first examined whether tHcy levels varied according to clinical diagnosis. ANCOVA of tHcy on diagnosis and sex, covarying age, found an association with diagnosis \( (p < 0.0001) \) (table, figure 1A), sex \( (p = 0.0051) \), and age \( (p < 0.0001) \). PD cases had higher tHcy relative to all diagnostic groups \((p \leq 0.0012)\), except...
p = 0.08 relative to hICH). The CAA group had marginally lower tHcy relative to AD and hICH (p < 0.03). Mean tHcy levels did not otherwise differ among AD, MCI, and non-demented control subjects. Men had higher tHcy levels than women, adjusted for age and diagnosis, and tHcy levels increased with age. tHcy did not correlate with duration or BDS-IMC in AD (figure 2A), although tHcy did positively correlate with markers of PD progression including duration of illness (p = 0.0008), BDS-IMC (p = 0.001), and H&Y stage (p = 0.002).

ANOVA of folate levels showed effects of age (p = 0.0003) and diagnosis (p = 0.02). Patients with AD had lower age-adjusted folate levels relative to MCI (p = 0.01), controls (p = 0.05), CAA (p = 0.01), and hICH (p = 0.01) (table). Folate levels increased with age.

Vitamin B12 levels were associated with diagnosis (p = 0.03), with lower vitamin B12 levels in PD relative to AD, MCI, and CAA (p ≤ 0.006) (table).

ANOVA of Cr levels showed effects of diagnosis (p = 0.04), age (p < 0.0001), and sex (p < 0.0001). The hICH group had higher Cr relative to all other diagnostic groups (p ≤ 0.03, except p = 0.06 for AD) (table); men had higher Cr levels than women, and Cr increased with age.

Patients with PD treated with levodopa had higher tHcy levels. We hypothesized that the elevated tHcy levels in PD were related to levodopa use. Evaluating tHcy in patients with PD taking levodopa and not taking levodopa, we found that the elevated mean tHcy levels within the overall PD group was primarily due to those taking levodopa. Levodopa use was associated with increased tHcy, corrected for age (n = 73, 11.21 ± 4.46 μmol/L) relative to patients with PD off levodopa (n = 20, 7.50 ± 2.31 μmol/L, p = 0.0001) (figure 1A). Patients with PD on both levodopa and catechol-O-methyl transferase inhibitor (COMT-I) (n = 17) had similar tHcy levels (11.18 ± 2.66 μmol/L) compared to patients with PD on levodopa alone (n = 56, 11.20 ± 4.91 μmol/L), suggesting that COMT-I does not further affect tHcy levels. In the entire PD group, tHcy correlated with levodopa dose (r[92] = 0.46, p < 0.0001), adjusted for age. Within the patients with PD on levodopa, tHcy also correlated with levodopa dose (r[72] = 0.36, p = 0.002), adjusted for age (figure 1B).

These data suggest that levodopa use may be driving the correlations in PD of tHcy levels with markers of disease progression and severity. To assess this possibility, we investigated correlations of tHcy with PD clinical measures, partialing age and levodopa dose. In the entire PD group, tHcy correlated with BDS-IMC (r[90] = 0.23, p = 0.03) (figure 2B), but not duration (r[90] = 0.14, p = 0.18) or H&Y (r[90] = 0.0, p = 1.0), adjusted for age and levodopa dose. In the patients with PD on levodopa, tHcy correlated with BDS-IMC (r[70] = 0.31, p = 0.01) but not duration (r[70] = 0.16, p = 0.18) or H&Y (r[70] = -0.07, p = 0.57), adjusted for age and levodopa dose. Therefore, within the PD group, tHcy appears to correlate with cognitive measures even after adjusting for levodopa dose, although we cannot rule out residual confounding.

Multivitamin use is associated with lower tHcy levels. tHcy levels are modifiable by vitamin supplementation. In the entire cohort, individuals taking multivitamins (n = 116) had lower tHcy levels (8.06 ± 2.93), higher folate levels (38.6 ± 27.4 nmol/L), and higher vitamin B12 levels (482.5 ± 208.8 pmol/L) compared with those not taking multivitamins (n = 282; tHcy, 9.61 ± 3.57 μmol/L, p < 0.0001; folate, 28.0 ± 22.6 nmol/L, p < 0.0001; vitamin B₁₂, 370.9 ± 190.5 pmol/L, p < 0.0001).

Biochemical associations. tHcy and folate, vitamin B₁₂, Cr. Elevated tHcy levels are often a marker of folate or vitamin B₁₂ deficiency. Covarying age, we investigated the correlation of tHcy values with other biochemical measures in plasma. In the entire sample, tHcy inversely correlated with folate (r[396] = -0.21, p < 0.0001), and vitamin B₁₂ (r[396] = -0.41, p < 0.0001), and positively correlated with Cr (r[396] = 0.39, p < 0.0001). Therefore, we detected the expected weak to moderate correlations between tHcy, folate, vitamin B₁₂, and Cr in our patient sample.

Correlations with plasma Aβ variables. Both homocysteine and Aβ are implicated in neurotoxicity, vascular toxicity, and AD risk. We tested for an association between
their plasma levels by partial correlation analysis of tHcy and Aβ variables, holding age constant. In the pooled AD, PD, MCI, and control cases, tHcy correlated with both Aβ40 (r[311] = 0.33, p < 0.0001) and Aβ42 (r[311] = 0.29, p < 0.0001) (figure 3, A through B). tHcy correlated with Aβ40 in each diagnostic group: AD (r[138] = 0.36, p < 0.0001), PD (r[92] = 0.26, p = 0.0144), MCI (r[35] = 0.40, p = 0.0177), and controls (r[85] = 0.31, p = 0.0042). tHcy correlated with Aβ42 in AD (r[138] = 0.35, p < 0.0001) and MCI (r[35] = 0.34, p = 0.0491), and controls (r[85] = 0.21, p = 0.0543), but nonsignificantly in PD (r[92] = 0.16, p = 0.1327). The correlations were weaker when individuals only under the age of 65 (n = 74) were considered within the entire pooled group (Aβ40 r[74] = 0.15, p = 0.20); Aβ42 (r[74] = 0.24, p = 0.04).

In the pooled CAA, hICH group, tHcy correlated with Aβ40 (r[85] = 0.41, p < 0.0001) but not with Aβ42 (r[85] = 0.07, p = 0.53) (figure 3, C through D). Thus, within the entire cohort, plasma tHcy levels correlated with age and plasma Aβ40 levels.

It is possible that the correlation of tHcy with Aβ is confounded by other biochemical measures, since folate, vitamins B₁₂, and Cr are associated with tHcy and these also may also be associated with plasma Aβ. However, folate and vitamin B₁₂ did not correlate with Aβ measures within the pooled AD, PD, MCI, and control cases or in the pooled CAA, hICH diagnostic group.

In the pooled AD, PD, MCI, and control cases, Cr correlated with Aβ40 (r[313] = 0.25, p < 0.0001) and Aβ42 (r[313] = 0.17, p = 0.0021); in the pooled CAA, hICH cases, Cr correlated with Aβ40 (r[85] = 0.38, p = 0.0003) but not with Aβ42 (r[85] = 0.14, p = 0.21). Therefore, there is the possibility of confounding by Cr. Nonetheless, the correlations between Aβ variables and tHcy persisted after correcting for Cr: tHcy correlated with Aβ40 (r[311] = 0.26, p < 0.0001 in AD, PD, MCI, controls and r[85] = 0.28, p = 0.01 in CAA, hICH), adjusted for age and Cr. tHcy correlated with Aβ42 (r[311] = 0.24, p < 0.0001) in the AD, PD, MCI, and control groups adjusting for age and Cr.

Genetic correlations. Genetic polymorphisms, diagnosis, and tHcy levels. The APOE ε4 allele was overrepresented in AD, MCI, and CAA cases relative to PD and control cases, but had no effect on tHcy levels (analysis of variance APOE genotype vs tHcy [p = 0.63]). The association of tHcy with Aβ40 and Aβ42 in the pooled AD, MCI, PD, and control cases and the association of tHcy with Aβ40 in the pooled CAA, hICH cases was not modified by the presence or absence of APOE ε4 allele (p > 0.47) or the APOE ε2 allele (p > 0.17).

Discussion. Elevated tHcy is an established risk factor for vascular disease, including ischemic heart disease and stroke.44 Several studies suggest that elevated tHcy is also a risk factor for leukoencephalopathy,35 cognitive impairment,5-7 and AD.8-11 However, other studies did not detect significant associations with AD or cognitive status,12-14 stressing the need for further careful study.

We investigated the clinical and biochemical correlates of tHcy concentrations in plasma from AD, PD, MCI, CAA, hICH, and nondemented control individuals. The principal clinical factors influencing tHcy levels were age, levodopa use, and multivitamin use. Biochemically, in addition to the expected correlations between tHcy levels with folate, vitamin...
B₁₂, and Cr, we found that tHcy levels moderately and independently correlated with plasma Aβ levels.

Several groups reported elevated tHcy in AD cases,⁸⁻¹¹ attributed to relative deficiencies of folate and vitamin B₁₂. While we found lower folate levels in our AD cohort, tHcy and vitamin B₁₂ were not influenced by an AD diagnosis. Mean tHcy levels did not discriminate among AD, MCI, and nondemented control groups in our study. tHcy was not associated with cognitive status in AD and MCI. Our data are consistent with those of Luchsinger et al.,¹⁴ who found that cross-sectional and longitudinal analyses of tHcy with prevalent and incident AD were significantly confounded by age, sex, education, and APOE ε⁴. Since our study is based on a single measurement of tHcy, subtle disease associations may be underestimated due to regression dilution or prevalence bias.⁶³ AD cases of short duration (<5 years) had similar mean tHcy levels (8.5 μmol/L) relative to cases with longer duration (8.8 μmol/L) and control cases (8.7 μmol/L) (p > 0.90), arguing against significant prevalence bias by early vascular death of AD cases with higher tHcy. Other potential reasons that our results differ from previous cross-sectional studies include sample size, distribution of tHcy values, and the patient population. Our study was sufficiently powered to detect a 0.4-SD difference among the AD, PD, and MCI and indeed detected the significant increase in PD cases. The prevalence of hyperhomocysteinemia (tHcy >14 μmol/L) was relatively low in this population, perhaps reflecting the effects of folate supplementation of the U.S. dietary grain supply beginning in 1998.²² There are surprisingly few North American studies examining tHcy levels in AD after 1998; the improved folate status and reduced prevalence of hyperhomocysteinemia appears to have modified the cross-sectional association of tHcy with AD in the United States.¹³ Our northeastern United States–based clinic population may not be generalizable to other populations; nonetheless, other genetic and epidemiologic measures in these groups are consistent with known risks for AD (less education in AD/MCI groups, increased APOE ε⁴ in AD and MCI).

Similar to previous studies,¹⁵⁻²¹ levodopa use was strongly associated with hyperhomocysteinemia. Patients with PD taking levodopa had almost 50% higher average tHcy levels than patients not taking levodopa. tHcy results from the metabolism of levodopa and dopamine by COMT, wherein S-adenosylmethionine serves as a methyl donor. The resulting S-adenosylhomocysteine, is rapidly catabolized to homocysteine.

High levels of tHcy are implicated in nigral oxidative damage and cognitive deficits in PD. Homocysteine potentiates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity in mice, and rotenone toxicity in cultured dopaminergic neurons.³⁷ Within our PD group, which excluded co-existent dementia, tHcy correlated with cognitive status, even controlling for levodopa dosage. The association of elevated tHcy with lower cognitive status by a global screening test in non-demented PD subjects supports the results of O’Suilleabhain et al.,²⁰ where patients with PD with elevated tHcy performed poorly on neuropsychological testing. Thus, tHcy, which was greatly increased in levodopa users relative to the other diagnostic groups, may contribute to the cognitive decline that can develop in PD. Multivitamin use was associated with lower tHcy, even in levodopa users. Therefore, the particularly high levels of tHcy associated with levodopa use, and the potential risk of cognitive impairment may be amenable to folate and vitamin B₆ and B₁₂ supplementation.

A main finding of ours was the robust correlation of tHcy with plasma Aβ levels in most diagnostic groups. Our results confirm and extend a previous report of a positive correlation between plasma Aβ₄₀ and tHcy levels in a cross-sectional survey of community-dwelling men.³⁸ Possible mechanisms for the association may be that tHcy elevates Aβ levels, that Aβ levels increase tHcy, or that both are increased by an unknown third factor. In our sample and in other studies, plasma Aβ₄₀, plasma Aβ₄₂, and tHcy were correlated with age²³⁻⁴¹ and with serum Cr levels.⁴² We cannot rule out that another factor may result in the accumulation of both tHcy and Aβ in the plasma; however, based on careful covariate analysis of our data, the association is not explained by diagnosis, age, Cr, folate, vitamin B₁₂, or APOE polymorphisms. Cell culture data support the possibility that tHcy may increase Aβ levels: Homocysteine enhances Aβ generation by upregulating a presenilin-interacting endoplasmic reticulum stress protein⁴³; deficient methylation upregulates presenilin gene function and Aβ generation.⁴⁴

Although the correlation of tHcy and Aβ was independent of diagnosis in this cross-sectional study, age-related correlated increases in tHcy and Aβ may contribute to neurotoxicity and AD risk. Homocysteine potentiates Aβ oxidative toxicity in cultured neurons and smooth muscle cells and in APP transgenic mice.³,⁴⁻⁴⁷ Elevated tHcy and elevated plasma Aβ levels are both implicated as premorbid risk factors for the development of AD¹¹,⁴⁰,⁴¹ and for associated microangiopathic changes on MRI.⁵,⁴⁸ Both tHcy and Aβ₄₀ could be markers of vascular damage in the brain; tHcy and Aβ₄₀ were associated with white matter ischemic changes in APOE ε⁴ carriers in the Rotterdam study.²⁵,⁴⁸ The more general association of tHcy with plasma Aβ is independent of diagnosis (both neurodegenerative and cerebrovascular) and APOE genotype in our sample.

Translating the plasma measures to the tissue levels in the brain is a challenge that will require further investigation. tHcy levels in plasma and CSF are correlated,⁴⁸ but plasma and CSF Aβ levels are not correlated in AD.⁶ The relationship of plasma Aβ levels to CSF and brain levels is complicated by the kinetics of the blood-brain barrier, differential synthesis within the brain and the periphery, and
the effects of Aβ deposition as amyloid deposits in the brain.50–55 Plasma tHcy and Aβ directly contact elements of the blood vessel wall, providing a potential mechanism for resulting vascular toxicity in CAA and leukoencephalopathy. Further longitudinal epidemiological studies and as well as basic research investigations can address whether neurotoxicity in AD and PD is potentiated by the dual elevation of both tHcy and Aβ or whether tHcy and Aβ are markers of pathogenic processes such as vasculopathy or oxidative damage.

References


Carotid dissection causing occipital lobe infarction

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A 57-year-old man with a history of mild hypertension and Parkinson disease experienced a sudden loss of vision on the right. Initial examination was notable for a right homonymous hemianopsia but was otherwise normal. MRI demonstrated a left occipital lobe infarction, a tapered, “flame-shaped” left internal carotid artery occlusion with mural hematoma, and a fetal origin of the left posterior cerebral artery from the left internal carotid artery (figure). The patient was treated with anticoagulant therapy.

Majority supply of the posterior cerebral artery from the internal carotid artery occurs in 15 to 40% of normal subjects; in 10%, the posterior cerebral artery is supplied exclusively from the internal carotid artery.1 In such cases, carotid dissection or stenosis can cause occipital lobe infarction.2 Recognition of this so-called “fetal posterior cerebral artery” can have major clinical importance in accurately determining stroke mechanism and choosing appropriate preventive therapy.

Disclosure: The authors report no conflicts of interest.
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Figure. (A) T2-weighted brain MRI reveals origin of the posterior cerebral artery from the internal carotid artery (arrow) and left occipital lobe infarction. (B) Gadolinium-enhanced MR angiography of the neck shows a tapered, “flame-shaped” left carotid occlusion. (C) Axial TI-weighted noncontrast images show mural hematoma (arrow) in the distal left internal carotid artery with reconstitution of flow likely on the basis of collateral supply.

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