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# Total Homocysteine Is Associated With White Matter Hyperintensity Volume

## The Northern Manhattan Study

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***Background***—Total homocysteine (tHcy) has been implicated as a risk factor for stroke and dementia, but the mechanism is unclear. White matter hyperintensities may be a risk factor for both, but studies of the relationship between tHcy and quantitative measures of white matter hyperintensity volume (WMHV) are lacking, especially in minority populations.

***Methods***—A community-based sample of 259 subjects with baseline tHcy levels underwent pixel-based quantitative measurement of WMHV. We examined the relationship between tHcy and WMHV adjusting for age, sociodemographics, vascular risk factors, and $B_{12}$ deficiency.

***Results***—Higher levels of tHcy were associated with WMHV adjusting for sociodemographics and vascular risk factors.

***Conclusions***—These cross-sectional data provide evidence that tHcy is a risk factor for white matter damage. **(*Stroke*. 2005;36:1207-1211.)**

**Key Words:** homocysteine ■ magnetic resonance imaging ■ white matter

Increasing evidence suggests that white matter hyperintensity lesion burden detected on MRI represent small-vessel disease,[1–3] increase the risk of stroke,[3,4] and are associated with cognitive impairment and dementia.[5,6] Vascular risk factors such as hypertension[7,8] and, to a lesser extent, diabetes[9,10] are associated with a greater lesion burden and there has been increasing interest in identifying potentially modifiable risk factors. One of these is the sulfur-containing amino acid homocysteine. Elevated total homocysteine (tHcy) has been associated with atherosclerotic disease and an increased risk of stroke and dementia.[11–14] Few studies have examined the effect of elevated tHcy in those with small-vessel disease.[15,16] However, white matter hyperintensities may be a marker of small-vessel disease and several studies have documented an association with elevated tHcy.[17–19] These data come from mostly white populations and there is limited understanding of the effect of elevated tHcy on white matter hyperintensities in blacks and Hispanics, who are at greater risk for hypertension, diabetes, and small-vessel disease.[20,21]

Few studies have used quantitative methods for measuring white matter hyperintensity volumes (WMHV), depending rather on semi-quantitative scales that are subject to limitations in inter-rater reliability.[8,22,23] Quantitative methods have been used, but the populations studied have been limited to the elderly, whites, or men only.[24,25] Evidence from Framingham supports an association between various vascular risk factors and quantitative measures of white matter hyperintensities, but tHcy was not included.[25] The Rotterdam study found an association between elevated tHcy and the presence of silent infarcts and white matter lesions using qualitative measures.[19] The purpose of this study was to determine the relationship of elevated tHcy to white matter hyperintensities using quantitative techniques in a stroke-free community-based population of Hispanic, black, and white subjects.

## Subjects and Methods

The Northern Manhattan Study (NOMAS) includes 3298 stroke-free participants identified through random digit dialing using dual-frame sampling to identify published and nonpublished numbers.[26] People were eligible if they never had a stroke diagnosed, were 40 years of age or older, and had been residents of Northern Manhattan for at least 3 months in a household with a telephone. Subjects from the telephone sample were recruited for in-person assessment and the overall response rate was 68%. Data were collected between 1993 and 2001 through interviews by trained bilingual research assistants using standardized data collection instruments, review of medical records, physical and neurological examinations by study physicians, and fasting blood samples for tHcy, glucose, and methylmalonic acid measurements (MMA). Standardized questions about vascular risk factors were adapted from the Centers for Disease Control and Prevention Behavioral Risk Factor Surveillance System as defined previously.[27] Hypertension was defined as a systolic blood pressure $\geq$140 mm Hg or a diastolic blood pressure $\geq$90 mm Hg based on the mean of 2 blood pressure measurements, self-report of a diagnosis of hypertension, or medical treatment thereof. Diabetes was defined as

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fasting blood glucose ≥127 mg/dL, self-report of a diagnosis of diabetes, or insulin or oral hypoglycemic use. Cardiac disease was defined as a history of coronary artery disease, atrial fibrillation, or myocardial infarction. Race–ethnicity was based on self-identification as described previously.[27] Changes in health or vital status were determined through annual telephone follow-up.

## Laboratory Assessments

Baseline fasting blood samples were drawn into serum tubes and spun within 1 hour at 3000*g* and 4°C for 20 minutes and immediately frozen at −70°C, shown to be stable for tHcy assays.[28] We measured serum tHcy and MMA levels using methods licensed for commercial use.[29] Vitamin $B_{12}$ deficiency was defined as an MMA level >271 nm/L.

## MRI Examination

We began enrolling subjects into the MRI substudy in 2003 using the following criteria: (1) age older than 55; (2) no contraindications to MRI; and (3) able to sign consent. There were 259 scans available for this analysis. Imaging was performed on a 1.5T MRI system (Philips Medical Systems, Best, the Netherlands) at the Hatch Research Center. Analysis of WMHV was based on a fluid-attenuated inversion recovery (FLAIR) image as is acquired in the Multi-Slice Turbo Spin Echo (MS-TSE) mode with a field of view of 250 mm, rectangular field of view of 80%, and an acquisition matrix of 192×133 scaled to 256×256 in reconstruction. The FLAIR image has a slice thickness of 3 mm with no gap, a echo time of 144 ms, a repetition time of 5500 ms, an inversion recovery delay of 1900 ms, and a flip angle of 90 degrees. Images were oriented parallel to a hypothetical line connecting the anterior commissure and posterior commissure.

For quantitative analysis of WMHV, MRI data were transferred to the University of California at Davis. Analyses were performed using the Quantum 6.2 package on a Sun Microsystems Ultra 5 workstation. All analyses were performed blind to subject personal identifying information.

White matter hyperintensity segmentation was performed in 2 steps according to previously reported methods.[30,31] Briefly, non-brain elements were manually removed from the image by operator guided tracing of the dura mater within the cranial vault including the middle cranial fossa, but excluding the posterior fossa and cerebellum. The resulting measure of the cranial vault was defined as the total cranial volume to correct for differences in head size among subjects. Inter-rater reliabilities for the MRI measures of intracranial volume (0.97), brain volume (0.97), and WMHV (0.99) from images of this study were high.

The first step in image segmentation required the identification of brain matter. Image intensity nonuniformities were then removed from the image and the corrected image was modeled as a mixture of 2 gaussian probability functions with the segmentation threshold determined at the minimum probability between these 2 distributions.[30,32] Once brain matter segmentation was achieved, a single gaussian distribution was fitted to image data and a segmentation threshold for WMHV was determined a priori as 3.5 standard deviations (SDs) in pixel intensity above the mean of the fitted distribution of brain parenchyma as described previously.[31] Morphometric erosion of 2 exterior image pixels was also applied to the brain matter image before modeling to remove the effects of partial volume cerebrospinal fluid pixels on white matter hyperintensity determination.

White matter hyperintensity volume was expressed as the proportion of total cranial volume to correct for head size and log-transformed to create a normal distribution (log-WMHV), which was used in all linear regression analyses. We also categorized WMHV, designating those values >1 SD above the age-predicted value (based on regression of WMHV on age) as WMHV-large.

## Statistical Analyses

The values for tHcy were log transformed to stabilize variance (log-tHcy). Total homocysteine levels >≈15 μmol/L have predicted incident stroke and were associated with lower cognitive scores compared with those below the mean in our larger cohort.[13,33] In the MRI study, the 90th percentile was lower (12.5 μmol/L). We therefore created 3 categories (tHcy-Tri): below the mean (9.2 μmol/L, reference group), between the mean and 1 SD, and >1 SD (Table 1). We used linear and logistic regression to examine the different measures of tHcy in relation to log-WMHV and WMHV-large. Univariate analyses using *t* tests and $\chi^2$ tests were used to examine the association between both measures of log-tHcy and both measures of WMHV and the following potential confounders: age (or age older than 65 years), sex, race–ethnicity, insurance status, a baseline history of current or past smoking, hypertension, diabetes, cardiovascular disease, $B_{12}$ deficiency (as defined), and creatinine level. Those with renal insufficiency were excluded (creatinine >1.5 mg/dL). We constructed multiple linear and logistic regression models with log-WMHV and WMHV-large as dependent variables. We included all variables associated with tHcy or logWMH at an $\alpha$ level <0.1.

**TABLE 1. Prevalence of Categorical Measures of tHcy and White Matter Hyperintensity Volume**

| tHcy Trichotomy | WMHV-Large | | No. |
|---|---|---|---|
| < 8.6 μmol/L | 107 | 32 | 139 |
| 8.6–11.9 μmol/L | 64 | 25 | 89 |
| >11.9 μmol/L | 17 | 14 | 31 |
| No. | 188 | 71 | 259 |

# Results

Our sample of 259 stroke-free participants was similar to the overall cohort (n=3298) but included more men (45% versus 37.2%), fewer participants with Medicaid (21.2 versus 33.8), and fewer with hypertension (64% versus 73%). The mean age was younger than the overall cohort (64.8 versus 69.2) and there were more participants younger than 65 (55.2% versus 65%). The race–ethnic distribution was essentially the same as the overall cohort (Hispanic 53.6%, black 60%, white 56%, missing 3%). The mean log-tHcy level was 10.0 μmol/L and the 90th percentile was 12.5 μmol/L. The number of subjects within the categories for tHcy and WMHV are presented in Table 1. Vitamin $B_{12}$ deficiency (MMA >271 nmol/L) was present in 9% of the sample compared with 14.7% of the overall cohort.

In univariate analyses, both log-tHcy (Table 2) and tHcy-Tri were positively associated with both measures of WMHV, age in years (and age older than 65), and male sex. Log creatinine was linearly associated with log-tHcy ($r$=0.32; $P$≤0.0001) but not with measures of WMHV. Participants with $B_{12}$ deficiency had mean tHcy values 3.3 points higher than those without, and those with tHcy values >1 SD above the mean were more likely to have $B_{12}$ deficiency (30% versus 5%). Log-WMHV was associated with age older than 65, female sex, and hypertension (Table 2). The prevalence of WMHV-large was greater in those with hypertension (31% versus 18%; $P$<0.05), and a trend was seen in those age older than 65 (32% versus 24%; $P$=0.1) and with cardiac disease (38% versus 25%; $P$=0.06). tHcy levels did not differ by race–ethnic group but the prevalence of WMHV-large was twice as high in blacks (40%) as in whites (20%) and Hispanics (24%). The prevalence of hypertension and cardiac disease did not differ by race–ethnicity.

**TABLE 2. Univariate Correlates of Log-tHcy and Log-WMHV**

| | Prevalence, No. (%) | tHcy μmol/L, Mean (SD) | WMHV mL, Mean (SD) |
|---|---|---|---|
| Age >65 | | | |
| Yes | 140 (54.1) | 9.3 (1.4)† | 0.56 (0.86)† |
| No | 119 (46) | 8.0 (1.4) | 0.28 (0.79) |
| Sex | | | |
| Women | 143 (55.2) | 8.0 (1.4) | 0.38 (0.09)† |
| Men | 116 (44.8) | 9.3 (1.4)† | 0.35 (0.09) |
| Race–ethnicity | | | |
| Hispanic | 134 (53.6) | 8.2 (1.4) | 0.36 (0.08) |
| Black | 60 (24) | 9.7 (1.5) | 0.40 (0.09)† |
| White (reference group) | 56 (22.4) | 8.7 (1.4) | 0.35 (0.08) |
| HS education | | | |
| Yes | 151 (58.3) | 8.6 (1.4) | 0.37 (0.09) |
| No | 108 (41.7) | 8.5 (1.5) | 0.36 (0.08) |
| Medicaid | | | |
| Yes | 55 (21.2) | 8.9 (1.4) | 0.37 (0.08) |
| No | 204 (78.8) | 8.5 (1.4) | 0.36 (0.08) |
| Hypertension | | | |
| Yes | 172 (66.4) | 8.6 (1.4) | 0.38 (0.09)† |
| No | 87 (33.6) | 8.4 (1.4) | 0.34 (0.08) |
| Diabetes | | | |
| Yes | 48 (18.5) | 8.4 (1.4) | 0.37 (0.08) |
| No | 211 (81.5) | 8.6 (1.4) | 0.36 (0.09) |
| Smoking history (current or past) | | | |
| Yes | 138 (53.3) | 8.6 (1.4) | 0.37 (0.09) |
| No | 121 (46.7) | 8.5 (1.4) | 0.36 (0.09) |
| Cardiac disease | | | |
| Yes | 48 (18.5) | 8.3 (1.3) | 0.37 (1.0) |
| No | 211 (81.5) | 8.6 (1.4) | 0.36 (0.09) |
| $B_{12}$ deficiency* | | | |
| Yes | 24 (9.3) | 11.6 (1.5)† | 0.36 (0.07) |
| No | 235 (90.7) | 8.3 (1.4) | 0.36 (0.09) |

*Methylmalonic acid >271 nm/L.

†Significant at $P<0.05$ (for race–ethnicity white is the reference group).

**TABLE 3. Multivariate Models of the Association Between Log-tHcy and tHcy-Tri With the Log White Matter Hyperintensity Volume (Log-WMHV) and the WMHV >1 SD (WMHV-Large)**

| | Log-WMHV | | WMHV-Large | |
|---|---|---|---|---|
| | Regression Coefficient | *P* | OR (95% CI) | *P* |
| Model 1‡ | | | | |
| Log-tHcy | 0.63† | <0.0001 | 3.1 (1.4–7.2)* | 0.01 |
| tHcy-Tri | | | | |
| >1 SD | 0.61** | 0.0005 | 3.0 (1.3–6.7)†† | 0.01 |
| Mean to 1 SD | 0.26 | 0.03 | 1.4 (0.8–2.7) | 0.26 |
| <Mean | Ref | . . . | Ref | . . . |
| Model 2§ | | | | |
| Log-tHcy | 0.47 | 0.003 | 3.0 (1.2–7.5) | 0.02 |
| tHcy-Tri** | | | | |
| >1 SD | 0.45 | 0.007 | 2.7 (1.1–6.6) | 0.03 |
| Mean to 1 SD | 0.16 | 0.17 | 1.2 (0.6–2.5) | 0.34 |
| <Mean | Ref | . . . | Ref | . . . |
| Model 3‖ | | | | |
| Log-tHcy | 0.46 | 0.003 | 3.1 (1.2–8.0) | 0.02 |
| tHcy-Tri | | | | |
| >1 SD | 0.46 | 0.006 | 2.9 (1.2–7.3) | 0.02 |
| Mean to 1 SD | 0.12 | 0.28 | (0.5–2.3) | 0.5 |
| <Mean | Ref | . . . | Ref | . . . |
| Model 4¶ | | | | |
| Log-tHcy | 0.50 | 0.002 | 4.2 (1.5–11.7) | 0.01 |
| tHcy-Tri | | | | |
| >1 SD | 0.52 | 0.003 | 4.0 (1.5–10.8) | 0.006 |
| Mean to 1 SD | 0.13 | 0.25 | (0.5–2.3) | 0.45 |
| <Mean | Ref | . . . | Ref | . . . |

*Odds ratio (OR) represents the odds on having WMHV-large per unit increase in log-tHcy.

†Regression coefficient represents the change in log-WMHV per unit increase in log-tHcy.

‡Model 1: tHcy or tHcy-Tri in relation to log-WMHV and WMHV-large.

§ Model 2: tHcy, age, sex, and race–ethnicity.

¶Model 4: model 3 plus $B_{12}$ deficiency.

‖Model 3: model 2 plus hypertension and cardiac disease.

**Regression coefficient represents the change in log-WMHV associated with having a tHcy level above one SD or between the mean and 1 SD, compared to below the mean.

††Odds ratio represents the odds on having WMHV-large associated with having a tHcy level above one SD or between the mean and 1 SD, compared to below the mean.

Log-tHcy level and the highest tHcy category compared with the lowest were both associated with log-WMHV and WMHV-large (Table 3, model 1). The association remained significant after adjusting for age, sex, and race–ethnicity (Table 3, model 2), for hypertension and cardiac disease (Table 3, model 3), and for $B_{12}$ deficiency (Table 3, model 4). Hypertension was positively associated with log-WMHV in the adjusted model ($P<0.05$) and the trend between cardiac disease and WMHV-large remained ($P=0.06$). Black subjects had higher log-WMHV compared with whites in the adjusted model ($P=0.01$) but the association with WMHV-large was a trend ($P=0.07$).

## Discussion

We found that tHcy levels >1 SD above the mean were associated with greater white matter hyperintensity volumes. Adjusting for vascular risk factors did not attenuate this effect, suggesting that tHcy is not simply a marker of vascular disease. Few subjects in this sample had tHcy levels considered pathological (>15 μmol/L), because the 90th percentile was 12.5 μmol/L. Lower values were caused by the younger age and lower prevalence of $B_{12}$ deficiency. Despite lower levels, values >1 SD above the mean log-tHcy (>11.9 μmol/L) were independently associated with both measures of WMHV compared with values below the mean. This argues against a threshold effect for tHcy.

This study involved Hispanic, black, and white subjects with lower socioeconomic status (21% with Medicaid) and educational attainment (42% less than high school) extending our

ability to generalize the findings. Black participants had a mean log-WMHV twice as high, and a much greater prevalence of WMHV-large (40%) than Hispanics (23.9%) or whites (19.6%). Black race was independently associated with log-WMHV in the adjusted model, although there was only a trend for WMHV-large ($P<0.07$). The prevalence of hypertension and cardiac disease in the sample was not higher in blacks and does not explain this difference. Further studies with a larger MRI sample may help clarify race–ethnic differences in WMHV. Greater tHcy levels in blacks have not resulted in a higher risk of vascular events or cognitive dysfunction in the overall cohort.[13,33] Actually, $B_{12}$ deficiency in blacks was one-half that in whites and Hispanics in the sample and overall, suggesting that blacks have higher tHcy levels for genetic or other reasons.

We acknowledge several limitations. The MRI sample is healthier than the overall cohort because of a survivor effect and the functional capacity required to come in for the study, but this would tend to bias our findings toward the null. Fasting tHcy was measured at baseline, raising concern that values were not representative of later levels. However, the intra-SD was smaller than the overall SD in those with 2 measurements.[13] Also, MRI scans were performed after folic acid fortification began in the US in 1998. Although tHcy levels were lower in those enrolled after 1998, we controlled for the year of collection and the results remained significant.[13] Total homocysteine levels were lower in the study sample than the overall cohort, but this would likely minimize any association with measures of WMHV. Regarding potential confounders of the relationship between tHcy and WMHV, study subjects were younger and were less likely to have $B_{12}$ deficiency than the overall cohort, but this again would tend to minimize any association.

That white matter hyperintensities on MRI represent small vessel damage has been shown by observational and pathologic studies[1–3] and is supported by the association of tHcy with WMHV in this sample. Recent data suggest it may do so by contributing to endothelial dysfunction.[16] The cross-sectional nature of this analysis does not allow a conclusion. Longitudinal imaging studies will be needed to clarify if elevated tHcy causes progression of white matter damage and whether it is on the causal pathway between elevated tHcy and outcomes such as stroke and cognitive decline.

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