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Serum homocysteine level is related to cerebral small vessel disease in a healthy population

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Neurology® 2019;92:e317-e325. doi:10.1212/WNL.0000000000006816

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Abstract

Objective

To evaluate the relationship between serum total homocysteine (tHcy) levels and cerebral small vessel disease (cSVD) in a healthy population.

Methods

We included consecutive participants who visited our department for health checkups between 2006 and 2013. We rated white matter hyperintensity volumes using both the Fazekas score and semiautomated quantitative methods. We also evaluated lacunes, cerebral microbleeds, and enlarged perivascular spaces (EPVS), which are involved in cSVD. To assess the dose-dependent relationship between tHcy and cSVD parameters, we scored the burdens of each radiologic marker of cSVD.

Results

A total of 1,578 participants were included (age 55 ± 8 years, male sex 57%). In the multivariable analysis, tHcy remained an independent predictor of the white matter hyperintensity volume (B = 0.209; 95% confidence interval [CI] = 0.033–0.385, p = 0.020), presence of cerebral microbleeds (adjusted odds ratio = 2.800; 95% CI = 1.104–7.105, p = 0.030), and moderate to severe EPVS (adjusted odds ratio = 5.906; 95% CI = 3.523–9.901, p < 0.001) after adjusting for confounders. Furthermore, tHcy had positive associations with periventricular Fazekas score (p = 0.001, p for trend <0.001), subcortical Fazekas score (p = 0.003, p for trend = 0.005), and moderate to severe EPVS lesion burden (p < 0.001, p for trend <0.001) in a dose-dependent manner.

Conclusions

Serum tHcy level is correlated with cSVD development in a dose-dependent manner. These findings provide us with clues for further studies of the pathophysiology of cSVD.

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Glossary

aOR = adjusted odds ratio; BBB = blood-brain barrier; CI = confidence interval; CMB = cerebral microbleed; cSVD = cerebral small vessel disease; eNO = endothelial nitric oxide; EPVS = enlarged perivascular space; MRA = magnetic resonance angiography; TE = echo time; tHcy = total homocysteine; TR = repetition time; WMH = white matter hyperintensity.

Cerebral small vessel disease (cSVD) encompasses a group of subclinical conditions with diverse pathologies (e.g., white matter hyperintensity [WMH], lacunes of presumed vascular origin, cerebral microbleeds [CMBs], and enlarged perivascular spaces [EPVS]).[1] Despite their different pathologic manifestations, cSVDs frequently coexist with each other, especially in the elderly.[2] Thus, these conditions may share a common mechanism. Many researchers have attempted to explain the above phenomenon based on findings such as diffuse hypoperfusion, endothelial dysfunction, and atherosclerosis.[3–5] However, the mechanism underlying the above finding remains unclear.

Serum total homocysteine (tHcy) is a sulfur-containing amino acid produced during methionine metabolism.[6] Elevated tHcy levels have been shown to be related to cardiovascular disease, Alzheimer dementia, and cerebrovascular diseases, suggesting endothelial dysfunction as a major mechanism.[6–10] Endothelial dysfunction has also been investigated as a major mechanism in the development of cSVD.[3,4] Thus, elevated tHcy levels may be associated with various cSVD components. However, most previous studies have only investigated the associations between tHcy levels and individual components of cSVD (especially WMH and lacunes) in populations with different characteristics.[7,9,11–14] To understand the effects of tHcy on each cSVD parameter in relation to the other parameters, as well as mutual interactions between the different parameters, one should study the different factors in the same homogeneous population.

High serum tHcy levels may be reversed by supplementation with a certain group of B vitamins (i.e., folate and vitamin B_{12}).[15,16] However, previous trials have failed to prove the benefit of such intervention in the prevention of ischemic stroke events.[17,18] It seems that following the occurrence of one ischemic stroke, disease progression might not be reversed by treatments to lower the level of tHcy. The main question might thus be whether or when preclinical cSVD lesions can be reversed or stopped from progressing with proper homocysteine-lowering treatment. Therefore, there is a strong need for studies focused on intermediate cSVD lesions. In this study, we aimed to evaluate the relationship between the serum tHcy level and cSVD. We also compared tHcy levels among individuals with each component of cSVD based on their burden in search of clues regarding the underlying pathomechanisms of cSVD.

Methods

Patients and population

We used a consecutive health checkup registry at the Seoul National University Hospital Health Promotion Center between January 2006 and December 2013 to select individuals aged between 30 and 70 years whose tHcy levels were recorded (n = 1,709). The health checkups included broad evaluations, including brain MRI, brain magnetic resonance angiography (MRA), and laboratory examinations.

We excluded participants who met the following criteria from the study: history of stroke or severe neurologic deficit (n = 56) and malignancies or severe hepatic or renal diseases (n = 75). A total of 1,578 neurologically healthy volunteers were finally included in the analyses.

Clinical assessment

All demographic and clinical factors, cardiovascular risk factors, and laboratory factors were broadly evaluated and included the following: age, sex, body mass index, hypertension, systolic and diastolic blood pressure, diabetes, hyperlipidemia, ischemic heart disease, current smoking, and use of antiplatelet medicine.[19] Laboratory examination results including glucose profile, lipid profile, and high-sensitivity C-reactive protein, creatinine, and tHcy levels were also evaluated after 12 hours of overnight fasting. To measure serum homocysteine levels, venous samples were collected in serum separation tubes and were separated immediately with centrifugation (1,920g for 10 minutes). Next, tHcy levels were measured in the serum using a chemiluminescent microparticle immunoassay within 2 hours of collection. All clinical and laboratory factors were fully assessed in all participants.

Radiologic assessment

In the current study, all participants who visited our center for a health checkup underwent both brain MRI and MRA using 1.5-tesla magnetic resonance scanners (Signa, GE Healthcare, Milwaukee, WI; or Magnetom Sonata, Siemens, Munich, Germany). We acquired the following broad MRI acquisitions: T1-weighted images (repetition time [TR]/echo time [TE] = 500/11 milliseconds [ms]), T2-weighted images (TR/TE = 5,000/127 ms), T2 fluid-attenuated inversion recovery images (TR/TE = 8,800/127 ms), T2 gradient echo images (TR/TE = 57/20 ms), and 3-dimensional time-of-flight MRA images (TR/TE = 24/3.5 ms, slice thickness = 1.2 mm). With the exception of the time-of-flight MRA images, the basic slice thickness was 5 mm in the axial plane.

While evaluating each component of cSVD, we rated WMH volume using both a computer-assisted semiautomated method in MIPAV (Medical Imaging Processing, Analysis, and Visualization) version 7.3.0 (NIH, Bethesda, MD) and a manual rating method based on the Fazekas scale.[19] Lacunes of presumed vascular origin were defined as asymptomatic,

well-defined lesions measuring 3 to 15 mm in size with the same signal characteristics as CSF on T2 or T1 MRI in the territory of a perforating arteriole.[1] CMBs were defined as focal round lesions smaller than 10 mm with low signal on T2 gradient echo images.[1] We separately rated lobar and deep CMBs, since they are thought to differ in pathophysiology.[5] We posited that lobar CMBs had a closer relationship with tHcy than deep CMBs, as they are known to result from endothelial dysfunction.[5] Thus, we classified the individuals with CMB lesions as follows: "lobar CMB" group, which included participants with CMB lesions only in lobar areas or in both lobar and deep areas, and a "deep CMB" group. The burdens of lacunes and CMBs were classified as absent, single, or multiple based on lesion number. EPVS was defined as a round, oval, or linear lesion smaller than 3 mm with a signal similar to that of CSF without a surrounding hyperintense rim.[1] EPVS in basal ganglia level are known to be closely associated with other cSVDs. Therefore, in line with previous studies, we classified the participants into groups with the following numbers of EPVS in the basal ganglia: 0–10, 11–25, and >25.[20,21] All radiologic assessments were rated by 2 well-trained neurologists (K.-W.N. and H.-Y.J.), and disagreements were resolved by discussion with a third rater (H.-M.K.).

Statistical analysis

Continuous variables with normal distributions are presented as mean (SD); those with nonnormal distributions are presented as median (interquartile range). Continuous variables with skewed data were transformed into a log scale. Only WMH volume was transformed into a squared root scale, as it had many zero values. In univariable analyses, we used simple linear regression analyses for WMH volume and simple logistic regression analyses for lacunes, CMBs, and EPVS to determine whether they are potential predictors of cSVD. To conduct these analyses, we classified individuals in the 10–25 and >25 EPVS groups as the moderate to severe EPVS group.[20,21] Variables with p values <0.10 in the univariable analyses and for creatinine were introduced into the multivariable linear and logistic regression analyses, respectively. We performed additional multivariable analyses for CMB lesions. In these analyses, we used the outcome variables of lobar CMB and deep CMB to obtain detailed information regarding the relationship between tHcy level and CMB development.

To assess the dose-dependence of the relationships between tHcy and various cSVD markers, we compared mean tHcy levels among individuals with different burdens of each cSVD marker (WMH, lacunes, CMBs, and EPVS). Because of the small number of individuals with multiple lesions, CMB lesions were only compared in individuals with and without lesions. In this analysis, we used the Mann-Whitney U test, Kruskal-Wallis test, and Jonckheere-Terpstra test.

To compare the characteristics of groups with high and low tHcy levels, we dichotomized the whole cohort into a higher tHcy group and a lower tHcy group using the median value. We used the Student t test or the Mann-Whitney U test to analyze continuous variables, and the χ^2 test or Fisher exact test to analyze categorical variables. All statistical analyses in the study were performed using SPSS version 21 (IBM Corp., Armonk, NY). Values of $p < 0.05$ were considered statistically significant.

Table 1 Baseline characteristics of the cohort (n = 1,578)

	Total
Age, y	55 (50–61)
Sex, male	902 (57)
Hypertension	242 (15)
Diabetes	197 (12)
Hyperlipidemia	379 (24)
Ischemic heart disease	45 (3)
Current smoking	298 (19)
Antiplatelet medication	120 (8)
Systolic blood pressure, mm Hg	126 (115–137)
Diastolic blood pressure, mm Hg	76 (69–84)
Glucose, mg/dL	91 (84–100)
Total cholesterol, mg/dL	200 (177–224)
hs-CRP, mg/dL	0.04 (0.01–0.15)
Creatinine, mg/dL	0.90 (0.80–1.04)
Total homocysteine, μmol/L	9.6 (8.2–11.7)
WMH volume, mL	1.00 (0.20–2.50)
CMBs	59 (4)
CMBs involving lobar areas	15 (1)
CMBs involving only deep areas	44 (3)
Lacunes of presumed vascular origin	118 (7)
Enlarged perivascular space	
0–10	959 (61)
11–25	466 (29)
>25	153 (10)

Abbreviations: CMB = cerebral microbleed; hs-CRP = high-sensitivity C-reactive protein; WMH = white matter hyperintensity.
Data represent median (interquartile range) or n (%).

Standard protocol approvals, registrations, and patient consents

The current study was approved by the institutional review board at Seoul National University Hospital (1502-026-647).

Data availability

Data supporting the findings of this study are available from the corresponding author on reasonable request.

Results

Our study included 1,578 neurologically healthy participants (mean age 55 ± 8 years, male sex 57%). The median volume of WMH lesions was 1.00 (0.20–2.50) mL. The frequencies of lacunes, CMBs, and moderate to severe EPVS, which are manifestations of cSVD, were 59 (4%), 118 (7%), and 619 (39%), respectively. The median level of tHcy was 9.6 (8.2–11.7) μmol/L. The baseline characteristics of the cohort are displayed in table 1. The higher tHcy group had high frequencies of male sex, hypertension, diabetes, current smoking, moderate to severe EPVS, and higher levels of systolic and diastolic blood pressure, glucose, high-sensitivity C-reactive protein, and creatinine than the lower tHcy group (table 2).

In univariable and multivariable analyses, tHcy remained an independent predictor of WMH volume (B = 0.209; 95% confidence interval [CI] = 0.033–0.385, p = 0.020) (table 3), the presence of CMBs (adjusted odds ratio [aOR] = 2.800; 95% CI = 1.104–7.105, p = 0.030), and moderate to severe EPVS (aOR = 5.906; 95% CI = 3.523–9.901, p < 0.001) after adjusting for confounders. tHcy did not have a close relationship with lacunes (aOR = 0.978; 95% CI = 0.465–2.056, p = 0.953) (tables 4 and 5).

Fifteen participants (1%) were in the lobar CMB group and 44 (3%) were in the deep CMB group. tHcy remained an independent predictor of lobar CMBs (aOR = 12.037; 95% CI = 3.381–42.857, p < 0.001) (table 6) but not of only deep CMBs. Considering the small sample size and the possibility

Table 2 Baseline characteristics of patients with low and high tHcy levels

	Lower tHcy group, <9.60 μmol/L	Higher tHcy group, ≥9.60 μmol/L	p Value
No.	778	800	
Age, y	55 (49–60)	56 (50–62)	0.094
Sex, male	253 (33)	649 (81)	<0.001
Hypertension	100 (13)	142 (18)	0.007
Diabetes	79 (10)	118 (15)	0.006
Hyperlipidemia	174 (22)	205 (26)	0.137
Ischemic heart disease	20 (3)	25 (3)	0.508
Current smoking	77 (10)	221 (28)	<0.001
On antiplatelet medication	54 (7)	66 (8)	0.327
Systolic blood pressure, mm Hg	124 (113–135)	127 (118–138)	<0.001
Diastolic blood pressure, mm Hg	74 (68–82)	77 (70–86)	<0.001
Glucose, mg/dL[a]	90 (84–99)	91 (84–101)	0.045
Total cholesterol, mg/dL	200 (177–225)	200 (177–224)	0.544
hs-CRP, mg/dL[a]	0.04 (0.01–0.14)	0.06 (0.01–0.16)	0.002
Creatinine, mg/dL	0.81 (0.73–0.92)	1.00 (0.90–1.10)	<0.001
WMH volume, mL	0.91 (0.20–2.20)	1.10 (0.12–2.76)	0.057
CMB	26 (3)	33 (4)	0.412
CMB involving lobar areas	4 (1)	11 (1)	0.078
CMB involving only deep areas	23 (3)	25 (3)	0.845
Lacune of presumed vascular origin	55 (7)	63 (8)	0.543
Enlarged perivascular space			<0.001
0–10	564 (72)	395 (49)	
11–25	171 (22)	295 (37)	
>25	43 (6)	110 (14)	

Abbreviations: CMB = cerebral microbleed; hs-CRP = high-sensitivity C-reactive protein; tHcy = total homocysteine; WMH = white matter hyperintensity.
Data represent median (interquartile range) or n (%).
[a] These variables were transformed to log scales.

Table 3 Simple and multiple linear regression analyses between possible predictors and the squared root-white matter hyperintensity volume[a]

	Univariable analysis		Multivariable analysis[b]	
	B (95% CI)	p Value	B (95% CI)	p Value
Age	0.050 (0.045 to 0.056)	<0.001	0.049 (0.043 to 0.054)	<0.001
Sex, male	−0.010 (−0.109 to 0.088)	0.837	—	—
Body mass index	0.000 (−0.016 to 0.016)	0.970	—	—
Hypertension	0.365 (0.231 to 0.498)	<0.001	0.138 (0.004 to 0.272)	0.043
Diabetes	0.327 (0.180 to 0.473)	<0.001	0.121 (−0.017 to 0.258)	0.085
Hyperlipidemia	0.012 (−0.102 to 0.126)	0.838	—	—
IHD	0.247 (−0.045 to 0.539)	0.097	0.058 (−0.215 to 0.332)	0.676
Current smoking	−0.153 (−0.277 to −0.029)	0.016	0.006 (−0.114 to 0.126)	0.921
Antiplatelet medication	0.246 (0.063 to 0.429)	0.009	−0.081 (−0.259 to 0.097)	0.372
Systolic BP	0.010 (0.007 to 0.013)	<0.001	—	—
Diastolic BP	0.010 (0.006 to 0.015)	<0.001	—	—
Glucose[c]	0.549 (0.293 to 0.805)	<0.001	—	—
Total cholesterol	0.000 (−0.001 to 0.002)	0.547	—	—
hs-CRP[c]	0.019 (−0.013 to 0.051)	0.251	—	—
Creatinine	−0.124 (−0.398 to 0.151)	0.376	−0.140 (−0.420 to 0.141)	0.328
tHcy[c]	0.242 (0.074 to 0.409)	0.005	0.209 (0.033 to 0.385)	0.020

Abbreviations: BP = blood pressure; CI = confidence interval; hs-CRP = high-sensitivity C-reactive protein; IHD = ischemic heart disease; tHcy = total homocysteine.
[a] White matter hyperintensity volume was transformed to a square root scale.
[b] Adjusted with $p < 0.10$ in the univariable analysis (age, hypertension, diabetes, ischemic heart disease, current smoking, antiplatelet medication, and creatinine).
[c] These variables were transformed to log scale.

of overfitting, we conducted a backward stepwise regression analysis, which led to the same results as the analysis presented above (aOR = 9.380; 95% CI = 2.874–30.613, $p < 0.001$) (table 6).

Evaluation of the relationship between the tHcy levels and the burdens of the different manifestations of cSVD revealed a positive dose-dependent correlation between tHcy level and the modified Fazekas score in both periventricular ($p = 0.001$, p for trend <0.001) and subcortical ($p = 0.003$, p for trend = 0.005) areas. Participants who had larger EPVS lesions also displayed a close relationship between EPVS burden and tHcy level ($p < 0.001$, p for trend <0.001). Participants who had lobar CMBs ($p = 0.038$) but not those with only deep CMB lesions ($p = 0.814$) had higher tHcy values. There was no association between lacunes and the tHcy level ($p = 0.703$, p for trend = 0.896) (figure). The volume of WMH lesions ($p = 0.005$) and the number of EPVS lesions ($p < 0.001$) showed linear correlations with tHcy levels throughout the whole range (data available from Dryad, figure e-1, doi.org/10.5061/dryad.45r6n66).

Discussion

In this study, we found that tHcy levels were associated with cSVD in a neurologically healthy population. Because these positive associations were consistent in the analyses of WMH, CMBs, and EPVS in a dose-dependent manner, our findings may be indicative of the presence of a shared pathway among the different pathologies.

Increasing evidence indicates that an elevated tHcy level is directly and indirectly associated with endothelial dysfunction.[22–27] To understand the close relationship between the serum tHcy level and cSVD, we proposed several possible explanations related to endothelial dysfunction. One explanation for our observation may involve the loss of blood-brain barrier (BBB) function. An elevated tHcy level may inhibit endothelial nitric oxide (eNO) via production of reactive oxygen species or the accumulation of asymmetric dimethylarginine, which would in turn lead to functional suppression of the BBB.[15,27–30] tHcy can also destroy the BBB mechanically through inflammatory cascades (e.g., upregulation of matrix metalloproteinases and degradation of

Table 4 Univariable logistic regression analyses between possible predictors and CMBs, lacunes, and EPVS

	CMB		Lacune		Moderate to severe EPVS	
	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Age	1.069 (1.031–1.107)	<0.001	1.093 (1.064–1.123)	<0.001	1.118 (1.101–1.136)	<0.001
Sex, male	1.020 (0.603–1.726)	0.941	0.817 (0.561–1.190)	0.293	1.841 (1.494–2.269)	<0.001
BMI	1.046 (0.964–1.136)	0.280	1.016 (0.957–1.080)	0.599	1.067 (1.032–1.104)	<0.001
Hypertension	1.940 (1.062–3.545)	0.031	1.540 (0.968–2.450)	0.069	2.177 (1.650–2.872)	<0.001
Diabetes	1.642 (0.838–3.219)	0.148	1.023 (0.582–1.797)	0.938	1.634 (1.211–2.205)	0.001
Hyperlipidemia	0.980 (0.532–1.806)	0.980	0.792 (0.498–1.259)	0.324	1.044 (0.824–1.322)	0.722
IHD	0.578 (0.078–4.268)	0.591	1.214 (0.427–3.450)	0.715	1.976 (1.088–3.589)	0.025
Current smoking	0.476 (0.203–1.117)	0.088	0.759 (0.452–1.274)	0.296	0.782 (0.601–1.018)	0.068
Antiplatelet medication	1.395 (0.587–3.315)	0.451	1.879 (1.056–3.346)	0.032	2.406 (1.647–3.515)	<0.001
Systolic BP	1.023 (1.007–1.038)	0.004	1.013 (1.001–1.024)	0.030	1.021 (1.014–1.027)	<0.001
Diastolic BP	1.027 (1.004–1.051)	0.022	1.019 (1.002–1.036)	0.027	1.025 (1.015–1.034)	<0.001
Glucose[a]	2.288 (0.683–7.663)	0.179	1.521 (0.597–3.879)	0.380	2.108 (1.239–3.585)	0.060
Total cholesterol	0.999 (0.991–1.006)	0.737	0.996 (0.990–1.001)	0.110	0.997 (0.995–1.000)	0.070
hs-CRP[a]	1.091 (0.923–1.290)	0.309	1.061 (0.939–1.198)	0.341	1.143 (1.070–1.222)	<0.001
Creatinine	0.953 (0.219–4.136)	0.948	0.558 (0.192–1.616)	0.282	4.345 (2.428–7.776)	<0.001
tHcy[a]	2.260 (1.001–5.105)	0.050	1.030 (0.539–1.968)	0.928	6.721 (4.510–10.016)	<0.001

Abbreviations: BMI = body mass index; BP = blood pressure; CI = confidence interval; CMB = cerebral microbleed; EPVS = enlarged perivascular space; hs-CRP = high-sensitivity C-reactive protein; IHD = ischemic heart disease; OR = odds ratio; tHcy = total homocysteine.
[a] These variables were transformed to log scales.

claudin-5) or physical insults due to endoplasmic reticulum stress that cannot be overcome by the unfolded protein response.[22,24,28–30] Whether it is destroyed functionally or mechanically, breakdown of the BBB may lead to perivascular infiltration of toxic materials (e.g., protease, immunoglobulin, complement components, and cytokines) into neural tissues or the blockage of interstitial fluid clearance via the glymphatic pathway.[4,31,32] These events would in turn lead to the development of WMH or lobar CMBs/EPVS, respectively.

Chronic diffuse hypoperfusion might also underlie the association between the tHcy level and cSVD. eNO has attracted attention because it can not only regulate cerebral perfusion via autoregulation but can also prevent arteriosclerosis by inhibiting fibrosis and proliferation of vascular smooth muscle cells.[25,30,31,33,34] Thus, chronic hypoperfusion state and occlusion of small arterioles (arteriosclerosis) induced by inhibition of eNO may result in WMH or lacune development.[31] In addition, an elevated tHcy level may also lead to the accumulation of amyloid proteins. As mentioned above, tHcy may be associated with lobar CMBs, as it leads to decreased clearance of amyloid via the glymphatic pathway. Additional evidence indicates that tHcy increases β-amyloid formation in experimental models.[35,36] Of interest, consistent with the above findings, we observed a close relationship between higher tHcy level and the presence of lobar CMBs, which is thought to result from amyloid angiopathy.[1,5] This association was absent in the deep CMB group, providing further evidence for known differences in the pathologies of lobar and deep CMBs.[1] Finally, our observations may be attributable to simple coincidences stemming from shared risk factors in individuals with higher tHcy levels and cSVD. tHcy level is associated with age, hypertension, smoking, renal function, and other cardiovascular risk factors, which are also risk factors of cSVD development.[16] These risk factors may thus simultaneously lead to subclinical cSVD and elevation of the serum tHcy level.

In this study, we did not observe a correlation between lacunes and the serum tHcy level. Because an elevated tHcy level may induce atherosclerosis or prothrombotic conditions (e.g., platelet aggregation; activation of factors V, X, and XII; and inhibition of protein C and thrombomodulin),[28,37–39] we believed that it may have been related to lacune development, as reported previously.[7,9,12,14,40,41] The unexpected outcomes of the current study may be attributable to the characteristics of the participants. Our study included much younger healthy participants with a lower frequency of vascular risk factors and cSVD lesions.[12,14,38,40,41] According to the "two-hit model," which states that tHcy increases the risk of developing

Table 5 Multivariable logistic regression analyses between possible predictors and CMBs, lacunes, and EPVS

	CMB[a]		Lacune[b]		Moderate to severe EPVS[c]	
	aOR (95% CI)	p Value	aOR (95% CI)	p Value	aOR (95% CI)	p Value
Age	1.055 (1.017–1.094)	0.004	1.091 (1.061–1.121)	<0.001	1.121 (1.102–1.141)	<0.001
Sex, male	—	—	—	—	1.322 (0.964–1.813)	0.083
Body mass index	—	—	—	—	1.051 (1.010–1.094)	0.014
Hypertension	—	—	—	—	1.233 (0.882–1.722)	0.220
Diabetes	—	—	—	—	0.925 (0.656–1.305)	0.658
Hyperlipidemia	—	—	—	—	—	—
Ischemic heart disease	—	—	—	—	1.444 (0.717–2.907)	0.304
Current smoking	0.501 (0.205–1.227)	0.130	—	—	0.714 (0.519–0.984)	0.040
Antiplatelet medication	—	—	1.316 (0.726–2.382)	0.365	1.291 (0.823–2.024)	0.266
Systolic BP	1.019 (1.003–1.035)	0.023	—	—	—	—
Diastolic BP	—	—	1.021 (1.003–1.039)	0.023	—	—
Glucose[d]	—	—	—	—	—	—
Total cholesterol	—	—	—	—	0.997 (0.994–1.001)	0.124
hs-CRP[d]	—	—	—	—	1.065 (0.986–1.150)	0.109
Creatinine	0.580 (0.125–2.680)	0.485	0.573 (0.182–1.802)	0.340	1.575 (0.682–3.634)	0.287
tHcy[d]	2.800 (1.104–7.105)	0.030	0.978 (0.465–2.056)	0.953	5.906 (3.523–9.901)	<0.001

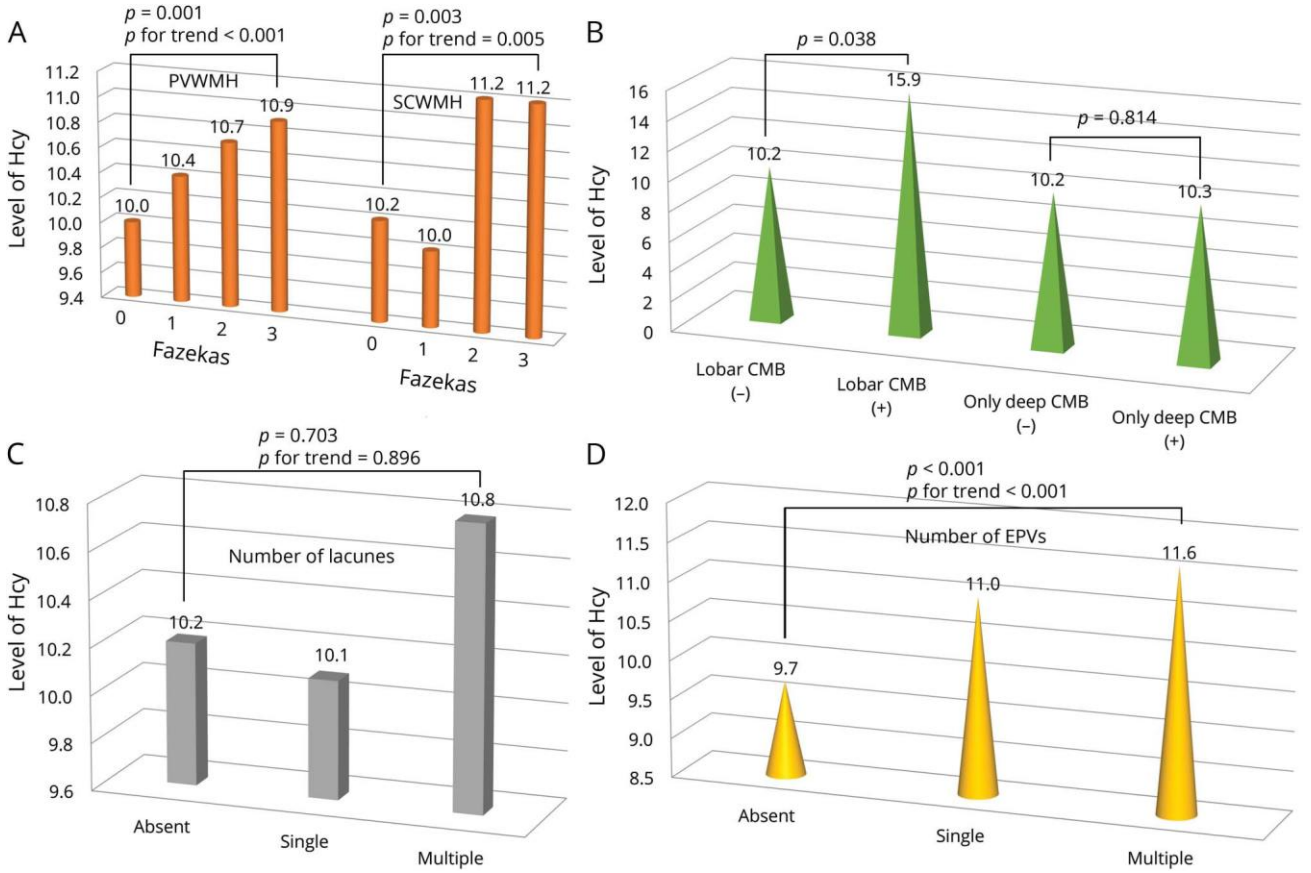
Abbreviations: aOR = adjusted odds ratio; BP = blood pressure; CI = confidence interval; CMB = cerebral microbleed; EPVS = enlarged perivascular space; hs-CRP = high-sensitivity C-reactive protein; tHcy = total homocysteine.
[a] Adjusted with $p < 0.10$ in the univariable analysis (age, systolic blood pressure, current smoking, and creatinine).
[b] Adjusted with $p < 0.10$ in the univariable analysis (age, diastolic blood pressure, antiplatelet medication, and creatinine).
[c] Adjusted with $p < 0.10$ in the univariable analysis (age, male sex, body mass index, hypertension, diabetes, ischemic heart disease, current smoking, antiplatelet medication, total cholesterol, hs-CRP, and creatinine).
[d] These variables were transformed to log scales.

Table 6 Multivariable logistic regression analyses between possible predictors and lobar/deep CMBs

	Lobar CMB		Deep CMB	
	aOR (95% CI)	p Value	aOR (95% CI)	p Value
Model 1				
Age	1.047 (0.976–1.123)	0.203	1.060 (1.016–1.105)	0.007
Systolic BP	0.988 (0.955–1.022)	0.481	1.029 (1.011–1.048)	0.002
Current smoking	0.399 (0.074–2.144)	0.284	0.548 (0.187–1.605)	0.273
Creatinine	0.960 (0.051–17.987)	0.978	0.685 (0.111–4.232)	0.684
tHcy[a]	12.037 (3.381–42.857)	<0.001	1.074 (0.317–3.638)	0.909
Model 2				
Age	1.058 (0.988–1.133)	0.109	1.064 (1.021–1.109)	0.003
Systolic BP	0.987 (0.954–1.022)	0.462	1.029 (1.011–1.048)	0.002
Creatinine	0.876 (0.048–15.918)	0.929	0.652 (0.106–4.009)	0.645
tHcy[a]	9.380 (2.874–30.613)	<0.001	0.929 (0.282–3.059)	0.903

Abbreviations: aOR = adjusted odds ratio; BP = blood pressure; CI = confidence interval; CMB = cerebral microbleed; tHcy = total homocysteine.
[a] These variables were transformed to log scales.

Figure Distribution of mean values according to the burdens of white matter hyperintensity volume, CMBs, lacunes, and EPVS



tHcytHcy had positive dose-dependent associations with periventricular Fazekas score (p = 0.001, p for trend <0.001), subcortical Fazekas score (p = 0.003, p for trend = 0.005), and moderate to severe EPVS lesion burden (p < 0.001, p for trend <0.001). Participants with lobar CMBs (p = 0.038), but not those with only deep CMB lesions (p = 0.814), had higher tHcy levels. There was no association between the tHcy level and lacunes (p = 0.703, p for trend = 0.896). CMB = cerebral microbleed; EPVS = enlarged perivascular space; PVWMH = periventricular white matter hyperintensity; SCWMH = subcortical white matter hyperintensity; tHcy = total homocysteine.

vascular lesions by exacerbating traditional risk factors,[7,28,39] younger age and a lower burden of vascular risk factors may diminish the association between tHcy and lacune via the ischemic pathway.

Previous studies have classified cSVD into at least 3 types: hypertensive small vessel disease in the deep area, called the "centrecephalon" (e.g., lacunes and deep CMB); venous disease in the periventricular area (e.g., periventricular WMH and EPVS); and a different form in the lobar regions where blood pressure is low (e.g., subcortical WMH and lobar CMB).[42,43] Of interest, our findings showed stronger association between tHcy and the last 2 cSVD types, supporting our hypothesis that tHcy is associated with cSVD via venous and arterial endothelial dysfunction. Meanwhile, tHcy did not show association with "hypertensive cSVD," which we considered as evidence that endothelial dysfunction has a lesser role in the development.

Although our study includes several novel findings and confirms the findings of previous studies, it was subject to some caveats. First, it was a single-center, retrospective, observational study in a single ethnic population. We had a large and relatively homogeneous sample population and conducted broad evaluations of all components of cSVD. However, the possibility of selection bias remains. Second, because of limitations of the cross-sectional analysis, we were unable to study causality. Future prospective studies are required to address this issue. Third, we did not consider other conditions that can affect serum tHcy levels. If we adjust for vitamin B levels or hypothyroidism, the relationship between tHcy and cSVD would be assessed more precisely. Fourth, because of the limitation of serum sampling, there may be a possibility of artifactual elevation of tHcy. To remove this effect, we attempted to minimize the duration that serum samples were at room temperature. However, this factor should be considered. Finally, because we included a healthy population, the numbers of individuals with CMB lesions was relatively small despite the large total sample size. However, our relatively healthy population had fewer vascular or neurologic problems, which may have impeded the study of the association

between a high tHcy level and various cSVD components. Our study thus enabled us to clearly assess independent associations between the tHcy level and the various cSVD parameters.

We demonstrated that an elevated serum tHcy level is independently associated with the development of cSVD, and especially with WMH, CMBs, and EPVS in a neurologically healthy population. Of note, these positive associations were observed within the tHcy levels recognized as traditionally "normal ranges" with a dose-dependent manner. Our findings thus provide us with clues for future studies of the pathophysiology of cSVD. However, our insights should be confirmed in larger prospective studies.

Author contributions

Concept and design: K.-W.N. and H.-M.K. Acquisition, analysis, or interpretation of data: K.-W.N., H.-Y.J., and S.-M.J. Drafting of the manuscript: K.-W.N., H.-M.K., and J.-H.P. Critical revision of the manuscript for important intellectual content: K.-W.N. and H.K. Statistical analysis: K.-W.N. and H.-M.K. Supervision: H.-M.K. and J.-H.P.

Study funding

No targeted funding reported.

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Publication history

Received by Neurology May 28, 2018. Accepted in final form September 27, 2018.

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