Homocysteine and Its Relationship to Stroke Subtypes in a UK Black Population
The South London Ethnicity and Stroke Study

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Background and Purpose—Homocysteine is an endothelial toxin and elevated levels have been associated with stroke risk. Stroke, particularly the small vessel disease (SVD) subtype, is increased in U.S. and UK black populations. In white populations elevated homocysteine has been associated with SVD, especially confluent leukoaraiosis, and may be acting through endothelial dysfunction. We determined the association between homocysteine and stroke subtypes, especially SVD, in a well-phenotyped UK cohort of black stroke patients compared to community controls.

Methods—Homocysteine, vitamin B12, folate levels, and renal function were measured in 457 black stroke patients recruited consecutively through the prospective South London Ethnicity and Stroke Study and 179 black community controls. All patients were subtyped using modified TOAST criteria. Leukoaraiosis in SVD patients was graded according to severity, and patients were additionally categorized on the basis of presence or absence of confluent leukoaraiosis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

Results—The highest homocysteine levels were seen in SVD patients compared to controls (16.2 [11.6] versus 11.8 [5.7] μmol/L, P<0.001) after adjusting for age, gender, vascular risk factors, vitamin levels, and renal function. Within SVD cases, highest homocysteine levels were found in lacunar infarction with confluent leukoaraiosis (19.6 [14.9] μmol/L) compared to lacunar infarction without leukoaraiosis (13.6 [7.1] μmol/L, P=0.001) and controls (P<0.001). Homocysteine correlated with leukoaraiosis severity (r=0.225, P<0.001).

Conclusions—In this well characterized UK black stroke population homocysteine levels were elevated and highest levels were found in lacunar stroke with leukoaraiosis. (Stroke. 2008;39:2943-2949.)

Key Words: stroke • homocysteine • leukoaraiosis

Severe hyperhomocysteinaemia, a feature of inborn errors of methionine metabolism, is associated with atherosclerosis.1 In addition, many studies have reported more modest increases in serum homocysteine in cardiovascular disease, with meta-analyses finding independent graded associations with coronary artery disease and stroke.2,3 The mechanisms underlying these associations are incompletely understood. Homocysteine is a prothrombotic factor affecting coagulation and fibrinolytic cascades.4 A key early event in homocysteine-mediated vascular damage is endothelial injury through direct toxicity and apoptosis,5 and endothelial dysfunction resulting in impaired endothelium-dependent dilatation of blood vessels combined with a proinflammatory and proatherosclerotic endothelial phenotype.6

Stroke is a heterogenous condition, and different stroke subtypes have different pathophysiological mechanisms. Homocysteine has been studied in stroke subtypes in various Asian and European populations.7–14 Although most studies suggest high homocysteine in all stroke subtypes compared with controls, some studies report stronger associations with particular subtypes including cerebral small vessel disease (SVD),7,8 large vessel disease (LVD),9–11 and primary intra-cerebral hemorrhage (PICH).13 The pathogenesis of SVD is incompletely understood but appears to be distinct from atheroembolism seen with LVD with differing risk factor profiles.15 Pathological and clinical studies have led to the hypothesis that there are two SVD subtypes: single larger lacunar infarcts (isolated lacunar infarction) attributable to microatheroma at the origins of larger perforator arteries (200 to 800 μm diameter), and multiple smaller lacunar infarcts with leukoaraiosis attributable to a diffuse arteriopathy affecting smaller perforator arteries (40 to 200 μm diameter).16,17 Endothelial dysfunction has been implicated in the leukoaraiosis variant of SVD, a subtype referred to as
ischemic leukoaraiosis. Elevated homocysteine has been reported in this SVD subtype in white populations, the association disappearing after controlling for circulating endothelial markers, implicating a role for endothelial dysfunction.

The incidence of stroke is increased in black compared to white populations in both the United States and United Kingdom. The distribution of subtypes also varies with an increased proportion of SVD in black stroke patients. Attenuated endothelial function has been reported in healthy stroke-free black volunteers, relative to whites. Homocysteine has been identified as a risk factor for ischemic stroke in whites but not black Americans in a prospective study where stroke subtyping was not performed. Whether elevated homocysteine is particularly associated with the SVD subtype in black populations is uncertain.

In this study, we determined the association between homocysteine and stroke subtypes in a well phenotyped UK cohort of black stroke patients compared to community controls. To explore possible associations with SVD further we compared homocysteine levels between the two SVD subtypes, and correlated levels with leukoaraiosis severity.

Methods

Study Population

The South London Ethnicity and Stroke Study is a prospective study that began in 1999 recruiting consecutive black stroke patients from a contiguous catchment area covered by three hospitals in South London. All hospitals have a specialist stroke unit and outpatient TIA clinics. Cases were recruited from both of these sources. In addition the prospective community based South London Stroke Register is nested within the catchment area and cases were also identified from this register. Over the period until 2006, 600 black stroke patients were recruited. Of these, 457 patients were included in this study. Of the remaining 143 patients, 58 (9.7%) had no blood taken (12 did not consent, 25 died before phlebotomy and 21 had difficulty with phlebotomy), in 59 (9.8%) the homocysteine assay did not produce a result (because of insufficient sample volume), and 26 patients (4.6%) were on folic acid or vitamin B12 supplementation were excluded because of effects on homocysteine levels.

All patients included in this study underwent brain imaging (CT alone, 282 [61.7%]; MRI with or without CT, 175 [38.3%]) and ECG. Imaging of the extracranial cerebral vessels with Duplex ultrasound, CT contrast angiography, or MR angiography was performed in 97.3% of ischemic strokes, and echocardiography in 55.2%. One hundred seventy-nine age- and sex-matched black patients were recruited for each subtype. Patients with stroke were enrolled only if they were living within the study area. Black ethnicity was defined as black or black British (black Caribbean or black African) according to the National Statistics classification used in the 2001 census in England and Wales (http://www.statistics.gov.uk/about/Classifications/ns_ethnic_classification.asp). The study protocol was approved by local research ethics committees, and informed consent was obtained from all participants.

Risk Factors

Risk factor information and other clinical and investigation details were collected on a standardized proforma. Hypertension was defined as pretreatment with antihypertensive drugs or a systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg persisting at least a week after stroke onset to exclude acute elevation of blood pressure attributable to stroke. Diabetes mellitus was defined as a previous diagnosis of type I or type II diabetes, or at least two random glucose readings of >11.1 mmol/L or fasting blood glucose readings of >7.0 mmol/L after the acute phase of stroke. Hypercholesterolaemia was defined as a serum cholesterol >5.2 mmol/L or prestroke treatment with a cholesterol-lowering agent. Smokers were defined as currently smoking or ever smoked.

Stroke Subtyping

One investigator subtyped all strokes with review of original brain imaging. A modification of the TOAST (Trial of Org 10172) subtyping classification was used. To avoid bias attributable to different rates of risk factors such as hypertension between the two groups, the presence of hypertension or diabetes was not used as a criterion in the diagnosis of SVD. SVD was defined as a clinical lacunar syndrome with a compatible lesion on MRI or CT or no lesion on CT without another cause of stroke identified. Exclusion criteria included subcortical infarction >15 mm diameter, cortical infarction of any size, carotid, vertebral, or intracranial artery stenosis >50%, and a potential cardiac source of embolism. Of the 152 SVD cases, 144 had a lacunar infarct visualized on CT or MRI, whereas 8 had a lacunar syndrome but no visible infarct on CT and no MRI performed. Large vessel atherosclerotic disease (LVD) was defined as carotid, vertebral, or major intracranial artery stenosis >50%. Cardioembolic stroke was defined based on the presence of a potential source of cardiac embolism categorized as high or moderate risk according to the TOAST criteria. Where no cause of stroke was found, patients were assigned into an “Unknown” category. Patients with more than one potential stroke mechanism was designated a “Tandem” category and rare causes of stroke were defined as “Other.” Primary intracerebral hemorrhage (PICH) was defined as a separate category. Patients with primary subarachnoid hemorrhage were excluded.

Leukoaraiosis Grading and SVD Subtyping

Leukoaraiosis on CT and MRI in all stroke patients was graded using the semiquantitative Fazekas scale which has been correlated with pathological severity in a postmortem validation study, modified to separate degrees of confluent leukoaraiosis as previously described: grade 0, no leukoaraiosis; grade 1, mild leukoaraiosis (>5 white matter hyperintensities); grade 2, moderate confluent leukoaraiosis; and grade 3, severe confluent leukoaraiosis. In addition, on the basis of this leukoaraiosis grade, SVD patients were subtyped into two groups: isolated lacunar infarction (lacunar infarction with absent or mild leukoaraiosis), or ischaemic leukoaraiosis (lacunar infarction in the presence of confluent leukoaraiosis) according to a previously validated method.

Biomarker Assessment

Nonfasting blood was collected from both patients and controls. Blood was centrifuged at 4400 r.p.m. and serum stored at −80°C. All biomarker assays were performed by the Department of Chemical Pathology, St George’s Healthcare NHS Trust blinded to subject identity. Homocysteine, B12, and folate were measured on serum using competitive immunoassay. Creatinine was measured on serum using direct colorimetry. Estimated glomerular filtration rate (eGFR) was calculated based on serum creatinine (μmol/L), age (years) and gender using the formula:

eGFR (mL/min/1.73 m²) = 175 × [(serum creatinine − 0.92)/(0.994)] × 0.0113121 ^{1.158} × (Age)^{−0.203} × 1.210 × (0.742 − if female)

Statistics

All statistical tests were performed using SPSS Version 15. The distributions of all biomarkers except eGFR were skewed and underwent natural logarithmic transformation to normalize distributions. Student t test was used for continuous variables and χ2 testing for categorical variables. Odds ratios (OR) and 95% confidence intervals per 1 μmol/L increase in log homocysteine were calculated using binary logistic regression analysis. Age, gender, hypertension,
Table 1. Demographic Details and Results of Biochemical Assays of Study Population

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=179)</th>
<th>Strokes (n=457)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, y</td>
<td>65.4 (7.4)</td>
<td>65.4 (12.2)</td>
<td>0.997</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>111 (62.0)</td>
<td>256 (56.0)</td>
<td>0.169</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>116 (64.8)</td>
<td>381 (83.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>47 (26.3)</td>
<td>186 (40.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypercholesterolaemia, n (%)</td>
<td>80 (44.9)</td>
<td>228 (51.8)</td>
<td>0.112</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>74 (41.3)</td>
<td>188 (41.4)</td>
<td>0.987</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>11.8 (5.7)</td>
<td>14.3 (8.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>B12, ng/L</td>
<td>779.2 (867.0)</td>
<td>718.3 (377.3)</td>
<td>0.446</td>
</tr>
<tr>
<td>Folate, μg/L</td>
<td>6.92 (3.05)</td>
<td>7.46 (3.96)</td>
<td>0.357</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m²</td>
<td>84.8 (24.9)</td>
<td>80.7 (30.5)</td>
<td>0.159</td>
</tr>
</tbody>
</table>

Homocysteine Comparison Between Stroke Patients and Controls

On univariate analysis homocysteine levels were elevated in stroke patients compared to controls (14.3 [8.9] μmol/L versus 11.8 [5.7] μmol/L, P=0.001; Table 1). This difference persisted after adjusting for age and gender (P<0.001), and after additionally adjusting for vascular risk factors, B12, folate, and eGFR (P<0.001). Homocysteine concentrations were separately elevated in both ischemic (14.3 [8.9] μmol/L) and hemorrhagic (14.5 [8.7] μmol/L) stroke compared to controls on univariate analysis (ischemic stroke, P=0.001; PICH, P=0.050), after adjusting for age and gender (ischemic stroke, P<0.001; PICH, P=0.005) and after additionally adjusting for vascular risk factors, B12, folate and eGFR (ischemic stroke, P<0.001; PICH, P=0.006).

Homocysteine levels in different stroke subtypes are shown in Table 2. Compared to controls homocysteine was elevated in all stroke subtypes, except LVD and “Other” categories, both before and after adjusting for age, gender, vascular risk factors, B12, folate, and eGFR. Highest homocysteine levels were seen in SVD. Homocysteine levels were higher in both SVD and cardioembolic stroke, compared to LVD on univariate analysis. After adjusting for age, gender, and vascular risk factors, B12, folate, and eGFR levels were significantly higher only in SVD.

Homocysteine and Stroke Risk

There was a graded positive relationship between homocysteine levels and stroke risk; OR per 1 μmol/L increase in log homocysteine was 1.93 (95% CI: 1.31 to 2.84). After adjusting for age and gender, the OR became 2.09 (95% CI: 1.40 to 3.12); after additionally adjusting for vascular risk factors, B12, folate, and eGFR, the OR was 4.02 (95% CI: 2.16 to 7.51; Table 3).

In a logistic regression model comparing homocysteine in all stroke patients and controls and after adjustment for age, gender, vascular risk factors, B12, folate, and eGFR, a significant interaction was observed between homocysteine and eGFR (P=0.004). To explore this further, the interaction was assessed in the same model using homocysteine tertiles. The significant interaction between homocysteine and eGFR occurred in the upper homocysteine tertile (P=0.044). Of all the homocysteine tertiles, the lowest mean eGFR (66.48 [31.17] ml/min/1.73 m²), equating to poorest renal function, was recorded in stroke patients in the upper tertile. However, the association between all strokes and homocysteine remained significant after adjusting for this interaction (P<0.001).

On analysis of association with stroke subtypes a graded positive relationship between homocysteine and risk was found for all stroke subtypes, except for LVD (Table 3). The adjusted OR was similar for hemorrhagic stroke (OR 4.65) compared to ischemic stroke (OR 4.02). This analysis was not performed for “Tandem” and “Other” categories because of the small numbers of patients in each subtype. The highest adjusted-risk was seen with SVD (OR 7.72) per 1 μmol/L log homocysteine. An interaction analysis demonstrated that the association between homocysteine and SVD was independent of age, gender, vascular risk factors, B12, folate, and eGFR.
Furthermore, the age- and gender-adjusted OR for the association between homocysteine and SVD did not change markedly on adding vascular risk factors to the regression model (data not shown).

**Homocysteine in SVD Subtypes**

There were significant differences between homocysteine concentrations in the two proposed SVD subtypes. Levels in ischemic leukoaraiosis (19.6 [14.9] μmol/L) were higher than those in both isolated lacunar infarction (13.6 [7.1] μmol/L; univariate P<0.001, and fully-adjusted P=0.013), and controls (univariate, P<0.001; fully-adjusted, P<0.001) Mean homocysteine in isolated lacunar infarction was significantly increased compared to controls (univariate, P=0.001; fully-adjusted, P<0.001). The OR per 1 μmol/L increase in log homocysteine for ischemic leukoaraiosis–control comparison was 17.10 (95% CI: 5.13 to 56.98), ischemic leukoaraiosis–isolated lacunar infarction comparison was 3.89 (95% CI: 1.52 to 9.96), and isolated lacunar infarction–control comparison was 5.65 (95% CI: 1.92 to 16.63) after full adjustment.

**Homocysteine Correlation With Leukoaraiosis Severity**

A significant positive correlation was seen between homocysteine and leukoaraiosis severity in all strokes (r=0.225, P<0.001) and SVD alone (r=0.256, P=0.001; Figure). After adjusting for age, gender, vascular risk factors, and vitamin status in an ANCOVA analysis, significant differences were seen between moderate (P=0.050) and severe (P=0.023) leukoaraiosis groups compared to the absent leukoaraiosis group in all strokes and between severe and absent leukoaraiosis groups (P=0.050) in SVD patients (Figure).

Pearson correlation coefficient (r) for the association between homocysteine and leukoaraiosis severity for all strokes, stratified to patients imaged with CT only was 0.182 (P=0.003) and stratified to MRI was 0.299 (P<0.001). For SVD, in patients with CT only r=0.241 (P=0.035), and in SVD patients with MRI imaging r=0.267 (P=0.021).

**Discussion**

This study found significantly elevated homocysteine levels in a UK black stroke population compared to community controls, and an independent graded association between homocysteine levels and stroke risk. Similar findings were demonstrated for both hemorrhagic and ischemic stroke. Within ischemic stroke cases homocysteine levels were elevated in all stroke subtypes with the exception of patients with LVD and strokes classified as “Other.” The strongest association was seen with SVD. The association was stronger in patients with the ischemic leukoaraiosis subtype of SVD, and there was a strong positive correlation between homocysteine levels and leukoaraiosis grade.

An independent, graded association has been demonstrated between homocysteine and stroke in prospective and retrospective studies in white populations. Causality is additionally supported by biologically plausible mechanisms for homocysteine-mediated vessel damage, and using the Men-
Hypertension, which is increased in black populations, is an important risk factor for SVD, but fails to account for all the risk. Endothelial dysfunction has been implicated in the pathogenesis of SVD. In white, markers of endothelial dysfunction are elevated in SVD especially in the presence of confluent leukoaraiosis. A similar association between homocysteine and confluent leukoaraiosis has been demonstrated; this association was attenuated after adjusting for markers of endothelial dysfunction suggesting that the pathogenic affect of homocysteine on small vessels be mediated by endothelial dysfunction. Endothelial function in healthy blacks is attenuated relative to healthy whites. In the current study, highest homocysteine levels were found in SVD patients who also had confluent leukoaraiosis.

A strong association was also found between homocysteine and PICH. Hemorrhage is increased in individuals with leukoaraiosis, and hypertensive PICH and SVD share similar pathology in the form of lipohyalinosis, in at least a portion of cases. It is possible that homocysteine-mediated small vessel damage may partly account for the association between homocysteine and PICH.

Strengths of this study included the prospective and consecutive recruitment of a well characterized cohort of African and African-Caribbean stroke patients and community controls. All patients had brain imaging and ECG, and almost all ischemic strokes had extracranial vessel imaging. Subtyping was performed by one rater with review of all original imaging using a pathophysiological method. Biochemical analyses were performed blinded to the stroke-control status to avoid bias. Homocysteine levels can be affected by vascular risk factors, vitamin status and renal function. These were adjusted for in analyses.

Differences in vitamin status and renal function failed to account for elevated homocysteine in black strokes, although a detailed dietary history was not recorded. A significant interaction between homocysteine and eGFR was seen especially at high homocysteine concentrations, but the association between homocysteine and stroke persisted after adjusting for this interaction. In young whites, genetic factors may be important.

Although our community control population was free of clinical cerebrovascular disease, brain imaging was not performed in this group. Therefore any relationship between silent brain infarction or asymptomatic leukoaraiosis and homocysteine could not be assessed. Scanning of controls would have markedly reduced their recruitment and could potentially introduce selection bias. If anything, by ensuring that controls were free of radiological cerebral ischemia, this would increase the magnitude of the demonstrated difference in homocysteine between patients and controls.

Patients with CT alone were included in the study to avoid selection bias. Although CT is less accurate than MRI in demonstrating the degree of leukoaraiosis, we have previously shown good agreement between grading of the degree of leukoaraiosis on this scale on CT and MRI, and analyses performed separately in CT-only and MRI groups dem-on...
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the results of HOPE-2 and VISP34 suggest a possible effect of homocysteine levels in all strokes (ischemic and hemorrhagic) compared to community controls. The strongest associations were seen with SVD. Within SVD, the highest homocysteine levels were seen in the presence of confluent leukoaraiosis. Coupled with the positive correlation between homocysteine and leukoaraiosis severity, this lends support to a pathogenic role for homocysteine in small vessel injury, possibly through endothelial cell dysfunction. Our findings may have important implications for the use of vitamin supplementation to lower homocysteine levels in the prevention of stroke. The large randomized-controlled secondary prevention trials of homocysteine lowering using vitamin supplementation such as NORVIT, HOPE-2, and VISP have failed to demonstrate a reduction of vascular events although a meta-analysis of these trials,29 and a recent reassessment of the results of HOPE-2 and VISP34 suggest a possible effect of vitamin therapy for stroke compared to myocardial infarction. Our results suggest that the efficacy of this therapy may differ for different stroke subtypes and be most beneficial in small vessel disease stroke. Such benefits will only be detected in trials which include adequate stroke subtyping. There may also be ethnic differences in efficacy, and sufficient black stroke patients should be included in trials to determine efficacy in this ethnic group.

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Disclosures

None.

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