Homocysteine is associated with hippocampus and white matter atrophy in older subjects with mild hypertension


Short title: Homocysteine and brain atrophy

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Abstract

Background: Plasma homocysteine has been associated with reduced brain volumes in cross-sectional studies. We aimed to investigate if homocysteine is associated with ongoing atrophy, and if so, if this is localised to grey or white matter.

Methods: In a group of 80 hypertensive subjects, aged 70-90 years, (from the SCOPE study) MRI images were obtained at 2 time points 2 years apart. Rates of grey and white matter and hippocampus atrophy were determined by calculating the difference in segmentation probability maps using SPM5. Plasma homocysteine, folate, B12 and creatinine were measured at study end.

Results: Homocysteine levels correlated with white matter atrophy rate (p = 0.006) hippocampal baseline volume (p =0.011) and hippocampal atrophy rate (p = 0.004) but not global grey matter atrophy or baseline grey or white matter volumes. The correlations remained significant (p < 0.05) after controlling for subject age, blood pressure, folate levels and white matter lesion volume.

Conclusion: In older hypertensives, plasma homocysteine levels are associated with increased rates of progressive white matter and hippocampal atrophy.

Keywords: brain atrophy, MRI, homocysteine, hippocampus, dementia
Introduction

Homocysteine is a sulphur containing non-essential amino acid, which plays an important role in cerebral metabolism. Homocysteine levels are inversely related to folate and B12 concentrations due to the role these vitamins play in homocysteine metabolism.

Homocysteine has neurotoxic effects through a variety of potential mechanisms (Obeid and Herrmann, 2006; Sachdev, 2005) including excitotoxicity, vascular changes & accelerating Alzheimer pathology. High levels of homocysteine have been found to be a risk factor for dementia. (Ravaglia et al., 2007; Seshadri et al., 2002) In studies of community dwelling older individuals, increased homocysteine has been associated with prevalence of white matter lesions in most studies (den Heijer et al., 2003; Sachdev et al., 2004; Vermeer et al., 2002), though not all (Longstreth et al., 2004). Presence of cerebral atrophy has also been observed in some (den Heijer et al., 2003; Whalley et al., 2003) but not all studies. (Longstreth et al., 2004; Sachdev et al., 2004) An association between raised homocysteine and hippocampal atrophy has also been reported (den Heijer et al., 2003; Williams et al., 2002). The reported associations with whole brain volume have all been done through cross-sectional studies, mostly assessed through visual ratings which are less sensitive than volumetric or longitudinal studies.

The aim of this work was to examine the relationship between homocysteine and brain atrophy over a 2 year period in a group of community dwelling individuals with hypertension. In a separate publication we show in our subjects a significant association between longitudinal rate of whole brain atrophy and homocysteine level (Narayan et al., submitted). In this article we examine the data to determine if there is a specific association
between HCys and the location of atrophy. We hypothesised that because white matter is more vulnerable than grey matter to ischaemic and hypoxia, white matter would be more vulnerable to the excitatory and vascular damage produced by homocysteine and that homocysteine would be more closely related to white rather than grey matter atrophy. In addition, we wished to see if our data confirmed the previous associations between homocysteine and hippocampal atrophy.

**Methods**

Hypertensive individuals were recruited to the Newcastle MRI substudy from one research centre participating in the larger international Study on COgnition and Prognosis in the Elderly (SCOPE). (Saxby et al., 2008) At entry to the main SCOPE trial, participants were aged 70-89 years, with systolic blood pressure (SBP) 160-179 mmHg and/or diastolic (DBP) 90-99 mmHg, untreated or thiazide-treated. Individuals were cognitively screened at entry to the trial, and those with possible dementia as defined by a Mini-Mental State Examination score <24 and/or who reported significant cognitive decline were not eligible. Those with MMSE in the range 24-27 were assessed using the Clinical Dementia Rating scale and the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) to exclude cognitive decline. The SCOPE study in total lasted four years. At the time of first MRI scan, hypertensive participants had been receiving treatment for an average of 24 months (range 13-34 months). The second MR scan was 710 days (range 694-721) after the first MR scan (ie 4 years after start of SCOPE study, and just before the end of the treatment). Blood samples were obtained at the end of the trial, an average of two weeks after the last MRI. Treatment was according to the published SCOPE protocol: (Hansson et al., 1999) essentially,
participants were randomised, double-blind, to treatment with the angiotensin receptor blocker, candesartan cilexetil, or placebo. Open label hydrochlorothiazide and additional antihypertensive therapy were permitted to achieve the target BP of <160/90 mmHg. Subsequently in 1999 target BP was changed to 150/90 mmHg at the Newcastle site in accordance with British Hypertension Society Guidelines. Blood pressure measurements were taken with three readings in the seated position after five minutes rest, with the mean of the second and third reading used. Blood pressure was measured at 6 monthly intervals, and we calculated the average of the BP readings between the two MRI scans.

Participants were invited to attend for MRI by telephone interview after screening for absolute contra-indications such as pacemaker or intraorbital foreign body. All participants gave informed, written consent. Ethical approval was granted by the Newcastle Joint Ethics Committee. 114 participants consented to and had a successful initial MRI scan. Repeat MRI scans were obtained from 97 people. Reasons for not repeating the MRI scan included refusal (n=10), moved out of the area (n=1), pacemaker fitted (n=1) and death (n=5).

_Homocysteine and related biochemical determinants_

At the end-trial visit, blood samples were drawn for total plasma homocysteine, vitamin B12 and folic acid. Non-fasting blood samples were obtained ensuring that the patients had not been in the lying/recumbent posture for an hour’s time prior to this (Rasmussen and Møllerer, 2001). The samples were collected in vacuotainers containing EDTA, put on ice immediately and centrifuged within 30 minutes at 4 degree Celsius to remove the cells from plasma. For
homocysteine assay, aliquots of the separated plasma were stored at -80 °C. Total plasma homocysteine was assayed by Abbot IMx Homocysteine assay system (Abbott Laboratories, Chicago, USA), based upon the fluorescence polarization immunoassay (FPIA) technology. This method has 95% limits of agreement of -0.97 to 2.30, an intralaboratory imprecision of less than 5% and showed linearity throughout the 6 to 50 μmol/l range. Plasma folate and B12 were measured by Abbott AxSYM analyzer. The coefficient of variation for folate assay was <10% for all levels. The B12 assay was by means of a micro particle based enzyme immunoassay, with a co-efficient of variation of <5% for medium or high levels, and <10% for levels of <100ng/l. (Lonati et al., 2004; Pernet et al., 2000). Normal ranges of these in our lab are: Serum folate 3.3-13.0 ug/L ; Creatinine 55 – 110 μmol/L; Homocysteine 5.4 - 11.5 μmol/L

**MRI acquisition**

All scans were performed on a 1.5 T GE Signa scanner (General Electric, Milwaukee, WI, USA). T1-weighted FSPGR (Fast Spoiled Gradient Recall) images were acquired in the coronal plane (TR=12.4ms, TE=4.2ms, TI=650ms, 256x256 matrix, slice thickness=1.7mm, flip angle=15°) yielding 124 slices through the brain. FLAIR (Fluid Attenuated Inversion Recovery, TR=10000ms, TE=142ms, TI=2100ms, slice thickness=5mm with 2mm interslice gap, 19 slices) and T2-weighted (TR=3420ms, TE=97.7ms, slice thickness=5mm with a 2mm interslice gap, 19 slices) were acquired in the axial plane. Participants were imaged twice with MRI scans two years apart (MRI-a and MRI-b), as part of a longitudinal study. The first scan (MRI-a) was performed approximately 2 years after entry into the main SCOPE trial. The same radiographer was used throughout, and images were aligned on each subject to be
parallel with a line joining the inferior most portion of the anterior splenium and posterior genu of the corpus callosum.

**MRI analysis**

Baseline and repeat MR images were processed using SPM5 (www.fil.ion.ucl.ac.uk/spm) with MATLAB 7.1 (The MathWorks, Inc. Natick, MA, USA). Default settings were used to segment images into grey and white matter and spatially transform all images to the space defined by the Montréal Neurological Institute (MNI). The grey and white matter maps produced represent the estimated probability of grey and white matter respectively in each voxel. Maps of grey and white matter loss were generated by subtracting the repeat probability maps from the baseline ones. To quantify the overall change separately in grey and white matter, firstly, masks were generated from each of the baseline grey and white matter maps using a threshold of 0.5 to identify all probable grey and white voxels, and then the mean of the subtracted image within this mask calculated for both grey and white matter. Brain tissue loss is thus represented by positive values of these variables.

I.e. if \( p_a(gm)_i \) is the probability of grey matter at pixel \( i \) at baseline and \( p_b(gm)_i \) the probability in the same pixel at repeat, then the total change in grey matter is given by the sum:

\[
\text{change in GM} = \sum_{\left[p_a(gm)_i > 0.5 \right]} (p_a(gm)_i - p_b(gm)_i)
\]
Rate of change of grey & white matter was calculated by dividing by the time (in years) between MRI scans and multiplying by 100 to give a percentage (of grey and white matter respectively).

Reliability of VBM for measuring white and grey matter volume changes was investigated by Shuter (Shuter et al., 2008) who found that (in the absence of signal-to-noise ratio (SNR) changes in the scanner) that there was no change in measured grey or white volume over a period of 2 months, and that the SD of the difference measurement was ~ 1.5 % of brain volume. We verified on 5 randomly selected subjects that the image SNR did not significantly change between first (mean 29.5 SD 5.3) and second scan (mean 30.5 SD 6.6). The SD of the grey and white matter difference measurement reported by Shuter et al is in agreement with our values.

Hippocampal volumes were measured from the baseline images using an automated segmentation technique (Firbank et al., 2008). Change in hippocampal grey matter was measured in a similar fashion to the whole brain grey matter change. The mask in this case was the same for all subjects - a region covering both hippocampi in MNI space. (Firbank et al., 2008)

The change values for GM, WM and hippocampus were all calculated in MNI space, and are thus corrected for cranial size. To adjust the baseline volumes of grey and white matter for head size, volumes were divided by the intracranial volume as estimated using the BET software (part of FSL http://www.fmrib.ox.ac.uk/fsl). Volumes of white matter lesions (WML) were measured from the FLAIR images using in-house semiautomated software as described previously. (Firbank et al., 2007)
Of the 97 who attended a second MRI scan, one subject did not have homocysteine measurements, 5 had motion artefacts, 1 subject with unsatisfactory brain segmentation and 10 were missing scans due to technical problems, leaving 80 subjects in the analysis. Of the 193 subjects with homocysteine measurements, there were no differences between those with vs without MRI in age, sex, MMSE or years of education. There were also no significant differences between those from whom successful repeat MRI scans were obtained and those from whom they were not.

Statistical analysis

We used the Kolmogorov-Smirnoff test to check for normality. Of the MRI variables, WML volumes were not normally distributed (p < 0.001) but all others (GM/WM/hippocampus volumes) were. In the whole sample, B12 (p 0.001) and homocysteine (p 0.006) creatinine ( p 0.005) were not normally distributed, whereas folate was. In order to obtain variables with a normal distribution, log values of B12, creatinine, and homocysteine and WML volumes were calculated. After log transformation, no variables were significantly non-normal. Pearson correlation coefficients were calculated between age and biochemical concentrations and MRI volumes and atrophy rates. We used partial correlation to investigate the association between MRI and homocysteine after controlling for confounding variables. Semi-partial (part) r values are quoted. All p values are 2 tailed.

SPM5 was used to perform voxel wise statistics to examine the relationship between the grey and white matter (baseline - repeat) difference maps and homocysteine, with additional
regression variables of subject age & folate levels. In addition, baseline (log) WML volume was included in the white matter regression. Parametric maps were thresholded at $p = 0.001$ uncorrected, and significance values are presented for clusters after controlling for multiple comparisons.

**Results**

Subject demographics are shown in table 1, and table 2 shows the imaging parameters for the whole group. The MMSE was measured also at study end, and there was no significant change. (paired t test $t = 0.9; p = 0.4$) All of the atrophy rates were significantly greater than zero. As table 3 shows, homocysteine strongly correlated with white matter atrophy ($p = 0.006$) and hippocampal atrophy ($p = 0.004$). It also correlated with baseline volume of the hippocampus ($p = 0.011$), but not grey or white matter volumes. Vitamin B12 levels did not show a correlation with MRI volumes or atrophy rates, and folate levels only a marginal correlation with baseline WM volume & creatinine with hippocampal atrophy rate. Average diastolic BP correlated with baseline GM volume, but otherwise BP did not relate to atrophy rate. In a semi-partial correlation, controlling for age, WML volumes and average BP, homocysteine still showed a significant relationship with WM atrophy rate ($r = 0.29; p = 0.010$), hippocampus volume ($r = -0.23; p = 0.035$) and hippocampal atrophy rate ($r = 0.32; p = 0.004$). Adding folate, B12 & creatinine to the model, Hcys was still significantly related to white matter atrophy rate ($r = 0.27; p = 0.016$), and hippocampal atrophy rate ($r = 0.22; p = 0.047$) however, hippocampus volume was no longer significant ($r = -0.19; p = 0.09$).

We also used SPM to investigate the location of correlations between HCys and grey and white matter loss. Figure 1 and table 4 show regions where WM atrophy rate correlates with
HCys, after controlling for age, baseline WML volume & folate levels. Controlling in addition for B12 and creatinine, the clusters in the corpus callosum and corona radiata were still significant, whereas the medial temporal lobe cluster no longer was. Figure 2 & table 5 show regions where WM loss correlates with baseline WML volume. As can be seen, this is in a different region to the HCys correlation & concentrated in a common location for WML. There were no significant clusters where GM loss correlated with HCys after controlling for age and folate, however we investigated change in the hippocampi using a small volume correction (10mm radius centred on the head of each hippocampus) and found a significant cluster on both hippocampi – see table 6. When covarying in addition for B12 and creatinine the right hippocampus cluster is still significant with a small volume correction.

Discussion

We found a strong relationship between homocysteine levels and both white matter and hippocampal rates of atrophy over two years. There was no significant relationship with whole grey matter loss. These findings add weight to previous cross-sectional studies of whole brain and hippocampal volumes.

The white matter loss occurred away from typical locations for WML, and separate from regions of white matter loss correlating with baseline WML. This suggests, in this group at least, that the HCys related atrophy of the white matter is not mediated through WML. This was also observed in the Rotterdam study (den Heijer et al., 2003) where their relationship between HCys and atrophy was not fully explained by WML, and the Framingham study (Seshadri et al., 2008) which found an association between HCys and silent brain infarcts, but not WML. This lack of association may be due to WML not being of purely vascular disease
origin, with pathological correlates including fluid accumulation and demyelination. (Fazekas et al., 1993)

Previous studies have shown an association between high HCys levels and hippocampal atrophy, both in normal populations (den Heijer et al., 2003; Williams et al., 2002) and in Alzheimer’s disease. (Clarke et al., 1998) The hippocampus is particularly vulnerable to ischemic injury (Back et al., 2004) and is affected early on in the course of Alzheimer’s disease, and high homocysteine levels have been associated with phosphorylated tau accumulation (Popp et al., 2009). Our findings that HCys levels were associated both with baseline and longitudinal atrophy of the hippocampus supports this evidence, pointing to a role of Hcys in the development of AD.

In line with a number of cross-sectional studies, we did not find a relationship between baseline grey or white matter volume and HCys. This is probably due to the number of other determinants of brain volume such as early nutrition and genetic factors (Peper et al., 2007) and shows the increased sensitivity of longitudinal studies.

The uniform appearance of white matter on T1 weighted images means that it is not possible to exactly locate atrophy, since atrophy in the centre of the white matter will show up as an enlargement of the nearest ventricle. Although grey matter atrophy might also be depicted as ventricular enlargement, it seems likely that the significant relationship is with white matter, since the homocysteine had a significant correlation with the global WM atrophy rate but not GM.
A potential weakness of this study is that homocysteine levels were only measured at the end of the study. However, previous longitudinal studies have found homocysteine levels relatively constant over 2 years (Clarke et al., 1998) and it is unlikely in this population without significant cognitive impairment that the changes in atrophy could have led to cognitive or behavioural changes that modified dietary folate intake. Prospective longitudinal studies of homocysteine and brain atrophy or cognitive decline would provide useful additional data to establish the causal relationships. Hypothyroidism has been associated both with raised homocysteine levels (Ozmen et al., 2006) and cognitive decline (Hogervorst et al., 2008), and may possibly have been a confounding factor. Unfortunately we did not measure thyroid stimulating hormone (TSH) levels.

In conclusion we have shown that rates of atrophy of white matter and hippocampus are related to high levels of homocysteine, independent of B vitamin levels or subject age. This adds to the research showing the neurotoxic effects of homocysteine.
Conflicts of interest

None

Description of Author Role

M.J. Firbank analysed the data and wrote the paper S.K. Narayan and B.K. Saxby assisted in analysis of the data and writing the paper. G.A. Ford designed the study, and supervised data collection. J.T. O'Brien designed the study, and supervised data analysis.

Acknowledgements

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References


Table 1 Demographic characteristics of subjects. Age and serum assays are at study end point. Weight and MMSE are at study start. BP is average between MRI scans. Values are mean ± SD [range]

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>79 ± 3.5 [74 - 91]</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>45/35</td>
</tr>
<tr>
<td>BP (Sys/Dias)</td>
<td>144/77 ± 12/6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71 ± 13 [44 – 113]</td>
</tr>
<tr>
<td>Years Education</td>
<td>10 ± 3 [5 – 16]</td>
</tr>
<tr>
<td>MMSE</td>
<td>29 ± 1 [26 – 30]</td>
</tr>
<tr>
<td>Serum B12 pmol / l</td>
<td>309 ± 107</td>
</tr>
<tr>
<td>Serum Folate µg / l</td>
<td>9.6 ± 3.5</td>
</tr>
<tr>
<td>Homocysteine µmol / l</td>
<td>14.8 ± 6.5</td>
</tr>
<tr>
<td>Creatinine µmol / l</td>
<td>99.9 ±22</td>
</tr>
</tbody>
</table>
Table 2. Imaging characteristics. WM = white matter. GM = grey matter. ICV = intracranial volume. Values are mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>One sample t test for atrophy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>WM (% of ICV)</td>
<td>31 ± 2.6</td>
</tr>
<tr>
<td>GM (% of ICV)</td>
<td>42 ± 2.8</td>
</tr>
<tr>
<td>Hippocampus volume (mm$^3$)</td>
<td>2480 ± 390</td>
</tr>
<tr>
<td>WM atrophy rate (% per year)</td>
<td>1.7 ± 0.7  ( p &lt; 0.001; t = 20 )</td>
</tr>
<tr>
<td>GM atrophy rate (% per year)</td>
<td>2.1 ± 0.9  ( p &lt; 0.001; t = 20 )</td>
</tr>
<tr>
<td>Hippocampal atrophy rate (% per year)</td>
<td>1.7 ± 1.4  ( p &lt; 0.001; t = 11 )</td>
</tr>
</tbody>
</table>
Table 3 Correlations between baseline and atrophy rate of grey and white matter & hippocampus, and age, B12, folate, homocysteine and average BP between MRI scans. B12 and Homocysteine are log values. Pearson r values are shown with 95% confidence intervals.

<table>
<thead>
<tr>
<th></th>
<th>Baseline white matter</th>
<th>Baseline grey matter</th>
<th>Baseline hippocampus volume</th>
<th>White matter atrophy rate</th>
<th>Grey matter atrophy rate</th>
<th>Hippocampal atrophy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td><strong>r (CI); p</strong></td>
<td><strong>r (CI); p</strong></td>
<td><strong>r (CI); p</strong></td>
<td><strong>r (CI); p</strong></td>
<td><strong>r (CI); p</strong></td>
<td><strong>r (CI); p</strong></td>
</tr>
<tr>
<td></td>
<td>-0.15 (-0.36 : 0.07) ; 0.2</td>
<td>-0.28 (-0.47 : -0.06) ; 0.013*</td>
<td>-0.33 (-0.52 : -0.12); 0.002**</td>
<td>0.25 (0.03 : 0.44); 0.028*</td>
<td>-0.15 (-0.36 : -0.07); 0.2</td>
<td>-0.09 (-0.31 : 0.13); 0.4</td>
</tr>
<tr>
<td><strong>B12</strong></td>
<td>-0.02 (-0.24 : 0.20); 0.8</td>
<td>0.0 (-0.22 : 0.22); 1.0</td>
<td>0.12 (-0.10 : 0.33); 0.3</td>
<td>-0.05 (-0.26 : 0.17); 0.7</td>
<td>0.07 (-0.15 : 0.28); 0.5</td>
<td>0.02 (-0.21 : 0.23); 0.9</td>
</tr>
<tr>
<td><strong>Homocysteine</strong></td>
<td>-0.15 (-0.36 : 0.07); 0.2</td>
<td>-0.09 (-0.30 : 0.13); 0.4</td>
<td>-0.28 (-0.47 : -0.07); 0.011*</td>
<td>0.30 (0.09 : 0.49); 0.006**</td>
<td>0.05 (-0.17 : 0.27); 0.6</td>
<td>0.32 (0.11 : 0.50); 0.004**</td>
</tr>
<tr>
<td><strong>Folate</strong></td>
<td>0.22 (0.00 : 0.42); 0.048*</td>
<td>-0.11 (-0.32 : 0.11); 0.3</td>
<td>0.14 (-0.08 : 0.35); 0.2</td>
<td>-0.17 (-0.38 : 0.05); 0.13</td>
<td>0.03 (-0.19 : 0.25); 0.8</td>
<td>-0.15 (-0.35 : 0.08); 0.2</td>
</tr>
<tr>
<td><strong>Creatinine</strong></td>
<td>-0.07 (-0.29 : 0.15); 0.5</td>
<td>0.07 (-0.16 : 0.28); 0.6</td>
<td>-0.10 (-0.31 : 0.12); 0.4</td>
<td>0.11 (-0.11 : 0.32); 0.3</td>
<td>0.03 (-0.19 : 0.25); 0.7</td>
<td>0.2 (0.02 : 0.43); 0.034*</td>
</tr>
<tr>
<td><strong>Diastolic BP</strong></td>
<td>0.08 (-0.15 : 0.29); 0.5</td>
<td>0.32 (0.10 : 0.50); 0.004**</td>
<td>0.03 (-0.19 : 0.25); 0.8</td>
<td>-0.13 (-0.34 : 0.09); 0.24</td>
<td>-0.05 (-0.27 : 0.17); 0.7</td>
<td>0.04 (-0.19 : 0.25); 0.8</td>
</tr>
<tr>
<td><strong>Systolic BP</strong></td>
<td>0.18 (-0.04 : 0.39); 0.1</td>
<td>0.1 (-0.12 : 0.31); 0.4</td>
<td>0.12 (-0.10 : 0.33); 0.3</td>
<td>0.0 (-0.22 : 0.22); 1.0</td>
<td>0.13 (-0.09 : 0.34); 0.25</td>
<td>0.13 (-0.09 : 0.34); 0.25</td>
</tr>
</tbody>
</table>

* p < 0.05  ** p < 0.01
Table 4 Significant clusters in SPM analysis of WM loss correlating with HCys, controlling for age, baseline WML volume & folate levels.

<table>
<thead>
<tr>
<th>Cluster size (voxels)</th>
<th>Cluster p_value (corrected for multiple comparisons)</th>
<th>Description of pixels in cluster</th>
<th>Location (MNI X,Y,Z mm)</th>
<th>Voxel Z statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>362</td>
<td>&lt;0.001</td>
<td>L corpus callosum</td>
<td>-14,-36,30</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L corona radiata</td>
<td>-26,-18,28</td>
<td>4.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L corpus callosum</td>
<td>-6,-32,24</td>
<td>4.3</td>
</tr>
<tr>
<td>58</td>
<td>0.051</td>
<td>Medial temporal lobe</td>
<td>-32,-28,-20</td>
<td>4.29</td>
</tr>
</tbody>
</table>
Table 5 Significant clusters in SPM analysis where WM loss correlates with baseline WML volume, controlling for age, folate & Homocysteine.

<table>
<thead>
<tr>
<th>Cluster size (voxels)</th>
<th>Cluster p_value (corrected for multiple comparisons)</th>
<th>Description of pixels in cluster</th>
<th>Location (MNI coordinates X,Y,Z mm)</th>
<th>Voxel Z statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>0.004</td>
<td>L posterior periventricular WM</td>
<td>-22,-54,24</td>
<td>4.61</td>
</tr>
<tr>
<td>50</td>
<td>0.09</td>
<td>R posterior periventricular WM</td>
<td>34,-50,24</td>
<td>4.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L corpus callosum</td>
<td>-6,-32,24</td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>0.051</td>
<td>medial temporal lobe</td>
<td>-32,-28,-20</td>
<td>4.29</td>
</tr>
</tbody>
</table>
Table 6 Significant clusters in the SPM analysis where GM loss correlates with homocysteine, controlling for age & folate. Small volume correction (10mm radius) centred on each hippocampal head.

<table>
<thead>
<tr>
<th>Cluster size (voxels)</th>
<th>Cluster p_value (corrected for multiple comparisons)</th>
<th>Description of pixels in cluster</th>
<th>Location (MNI coordinates X,Y,Z mm)</th>
<th>Voxel Z statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.025</td>
<td>L hippocampal head</td>
<td>-24,-12,-16</td>
<td>3.59</td>
</tr>
<tr>
<td>31</td>
<td>0.003</td>
<td>R hippocampal head</td>
<td>20,-14,-18</td>
<td>3.82</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1 Voxels where white matter atrophy rate shows significant correlation with HCys levels after controlling for age, baseline WML volume & folate. Cross-hair location -9,-29,29 mm. Overlaid on an average T1 weighted brain image.

Figure 2 Voxels where white matter atrophy rate shows significant correlation with baseline WML volume after controlling for age, baseline homocysteine & folate. Overlaid on an average T1 weighted brain image. Cross-hair location -22-54,24 mm