Plasma Total Homocysteine Levels and Cranial Magnetic Resonance Imaging Findings in Elderly Persons

The Cardiovascular Health Study

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Background: An elevated plasma total homocysteine (tHcy) level is associated with an increased risk of vascular disease. Some studies have shown associations between tHcy level and small-vessel disease of the brain on magnetic resonance imaging (MRI).

Design: In the Cardiovascular Health Study, 622 elderly participants without a history of transient ischemic attack or stroke had results for tHcy level and cranial MRI. We sought associations between tHcy level and MRI findings of ventricular grade, sulcal grade, white matter grade, and infarcts. We controlled for other factors, including levels of creatinine, folate, and vitamins B6 and B12 and methylenetetrahydrofolate reductase genotype.

Results: After controlling for age and sex, tHcy level was not associated with the individual MRI findings. Further adjustments for other factors and other blood tests had little effect on these findings. The only significant finding was a linear trend across quintiles of tHcy level and a pattern of MRI findings combining infarcts and high white matter grade. The linear trend remained significant after controlling for other risk factors and atherosclerotic markers (top quintile vs bottom quintile odds ratio, 3.3; 95% confidence interval, 0.96-11.20; \(P=0.04\) for linear trend) but was slightly diminished after further controlling for creatinine, folate, and vitamins B6 and B12, (odds ratio, 3.2; 95% confidence interval, 0.81-13.10; \(P=0.07\) for linear trend).

Conclusion: We were unable to confirm the results of previous studies with respect to tHcy level and individual MRI findings, although an association was seen for an MRI pattern combining infarcts and high white matter grade.

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plasma tHcy level was measured by high-pressure liquid chromatography and electrochemical detection, as previously described. Plasma vitamin concentrations were measured using radioimmunoassay. One system (Quantaphase II Assay System; Bio-Rad Laboratories, Hercules, Calif) was used for folate and vitamin B12, and a kit (3H-REA; ALPCO Diagnostics, Windham, NH) was used for pyridoxal phosphate, the bioactive form of vitamin B6. The DNA extracted from peripheral leukocytes was used to determine MTHFR C677T genotype, as previously described. Plasma assays were performed on blood samples that had not been previously thawed.

For these analyses, 58 of the 3660 participants who underwent MRI were excluded because they had a missing value for 1 or more of the MRI variables defined herein. Of the remaining participants, 722 had also been selected for the ancillary study and 2880 had not. Because 22 participants had missing values for tHcy, 700 remained with results for the MRI scan and tHcy level. Before MRI, 20 participants had a transient ischemic attack; 53, a stroke; and 5, both. The 78 participants with a history of transient ischemic attacks, strokes, or both and the 622 without such a history were more likely to be older white men than the 2880 who had undergone MRI but did not have tHcy level determined. Table 1 details these differences and others among the 3 groups. In general, the 78 participants had more prevalent disease and the 622 had less than the 2880. As part of an ancillary study, fasting blood specimens collected at the baseline examination of the original cohort were used to determine plasma levels of tHcy, folate, and vitamins B6 and B12 in 1300 participants (25.0% of the cohort): all those who had tHcy measurements available. Results were similar and conclusions the same whether we considered tHcy level as a continuous variable or as quintiles, with exceptions as noted. Following the approach used in the Rotterdam Scan Study, we used quintiles, which were based on all 1300 participants in the ancillary study. We examined the association between quin-
RESULTS

Several risk factors and biomarkers were significantly associated with tHcy level after controlling for age and sex, which were strongly and independently associated with tHcy level (Table 2). The strongest associations were for the biomarkers other than MTHFR genotype, which was not significantly associated with tHcy level.

Table 3 lists the associations between quintiles of tHcy level and MRI findings. Considering the individual MRI findings, ventricular and sulcal grades were significantly associated with tHcy level, but the significance was lost after adjusting for age and sex. Neither white matter grade nor infarcts were significantly associated with tHcy level before or after adjustment for age and sex.

The results concerning the MRI patterns defined in a previous cluster analysis are also given in Table 3. The linear trend across the quintiles of tHcy was significant.
only for the complex infarct cluster after adjustment for age and sex. Significance remained after adjustment for other risk factors (P = .03) and, in addition, for atherosclerotic markers but was slightly diminished after further adjustment for creatinine level, vitamin levels, and MTHFR genotype. In these analyses, when tHcy level was included as a continuous variable rather than as quintiles, all of the P values for the complex infarct cluster were smaller than those listed in the table for linear trend.

For the results presented in Table 3, we sought evidence and found none for significant effect modification (statistical interaction) by sex, baseline myocardial infarction status, the interval between obtaining blood sample and MRI, and MTHFR genotype (data not shown).
Also, none of the associations of MRI findings and tHcy level became significant after adjusting for other risk factors, atherosclerotic markers, creatinine level, vitamin levels, and MTHFR genotype, as individual groups of variables or in combinations (data not shown). Results of the analyses summarized in Table 3 were not substantively changed by controlling for the interval between obtaining blood sample and MRI, by focusing on participants with a tHcy level at the 90th percentile and above, or by including the 78 participants with a history of transient ischemic attack and/or stroke (data not shown).

COMMENT

Among these participants in the CHS, plasma tHcy level was not associated with individual MRI findings of white matter grade or infarcts, although a significant association was seen for an MRI pattern combining infarcts and high white matter grade. Plasma tHcy level was significantly related to ventricular and sulcal enlargement on MRI, but these associations lost their significance and the pattern of increasing grades for these MRI findings with increasing tHcy level was eliminated after adjustment for age and sex, both of which were strongly and independently associated with tHcy level. Finally, tHcy level was also not related to MRI-defined infarcts in participants who underwent 2 MRI scans separated by about 5 years, although small numbers (n=223) may have compromised these results. The lack of significant associations was not altered when multivariable models included, as groups or in combinations, other cerebrovascular risk factors, markers of atherosclerosis, or biomarkers, including creatinine, folate, vitamins B12, and MTHFR genotype. Results were similar regardless of whether the 78 participants with a history of transient ischemic attack, stroke, or both were included.

The only significant associations with tHcy level in the current analyses were for an MRI pattern combining infarcts and high white matter grade, the complex infarct cluster.10 In these analyses, the normal cluster, which included as groups or in combinations, other cerebrovascular risk factors, markers of atherosclerosis, or biomarkers, including creatinine, folate, vitamins B12, and MTHFR genotype. Results were similar regardless of whether the 78 participants with a history of transient ischemic attack, stroke, or both were included.

We were unable to confirm the findings from previous studies relating tHcy levels to individual MRI findings. Unlike other studies1,2,12,13 of symptomatic and asymptomatic subjects with MRI-defined infarcts, we could not demonstrate a significant association between tHcy level and either prevalent or incident MRI-defined infarcts. Similarly, we could not demonstrate an association with white matter grade, as was done in the Rotterdam Scan Study2 but not in other studies.12,14,15 In the Rotterdam Scan Study, the strongest association was for those with infarcts, severe white matter lesions, or both on MRI scan, which yielded an odds ratio of 3.0 (95% CI, 1.8-5.2) (n=1077) when comparing the top quintile of tHcy level with the bottom quintile. The trend across the quintiles of tHcy level was similar to what we observed in the complex infarct cluster, which yielded a similarly adjusted odds ratio of 3.0 (95% CI, 0.94-9.35) (n=622). Results limited to those with infarcts and severe white matter lesions were not provided in the Rotterdam Scan Study. Comparisons with results from the Rotterdam Scan Study are difficult because of unknown baseline vitamin supplementation in both cohorts and differences between the cohorts for known baseline characteristics, methods for blood collection and tHcy determination, time between blood collection and MRI, and measurement of white matter changes. Values for tHcy quintiles used in both studies were similar.

Despite its strengths, including an extensive examination and near complete follow-up, and a large number of participants, CHS has its limitations. Prime among them is selective and incomplete participation among those invited to undergo MRI. The overall good health of the participants chosen for these analyses may explain in part the difficulty in reproducing the findings of other studies. In addition, the time between blood testing and MRI was likely longer in CHS, slightly more than 3 years, while in other studies, although stratifying by this interval or controlling for this interval in the analyses had little effect. Perhaps results may have differed if the interval were smaller or if serial tHcy levels were available. The significant findings related to complex infarcts may reflect inadequate adjustment for confounding or may be due to chance given the number of comparisons we performed. Whether these findings apply to other racial groups cannot be addressed by this study because 96.6% of the participants were white. Finally, these issues would be better addressed by studies with a longitudinal rather than a cross-sectional design, as was used in this study.

We were unable to document an association between tHcy level and individual MRI findings, although a significant association was seen for an MRI pattern combining infarcts and high white matter grade. These findings provide some support for suggestions that an elevated tHcy level may cause subcortical vascular encephalopathy with diffuse white matter lesions and lacunes.16 Alternatively, prevalent vascular disease may predispose to an elevated tHcy level. Clinical trials17,18 are under way to evaluate whether vitamins given to patients with symptomatic cerebrovascular disease will reduce the risk of subsequent vascular events. Whether vitamin supplements would prevent small-vessel brain...
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For a full list of participating investigators and institutions in the CHS, see "About CHS: Principal Investigators and Study Sites," available at: http://chs3.chs.biostat.washington.edu/chs/.

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REFERENCES


