Homocysteine and stroke

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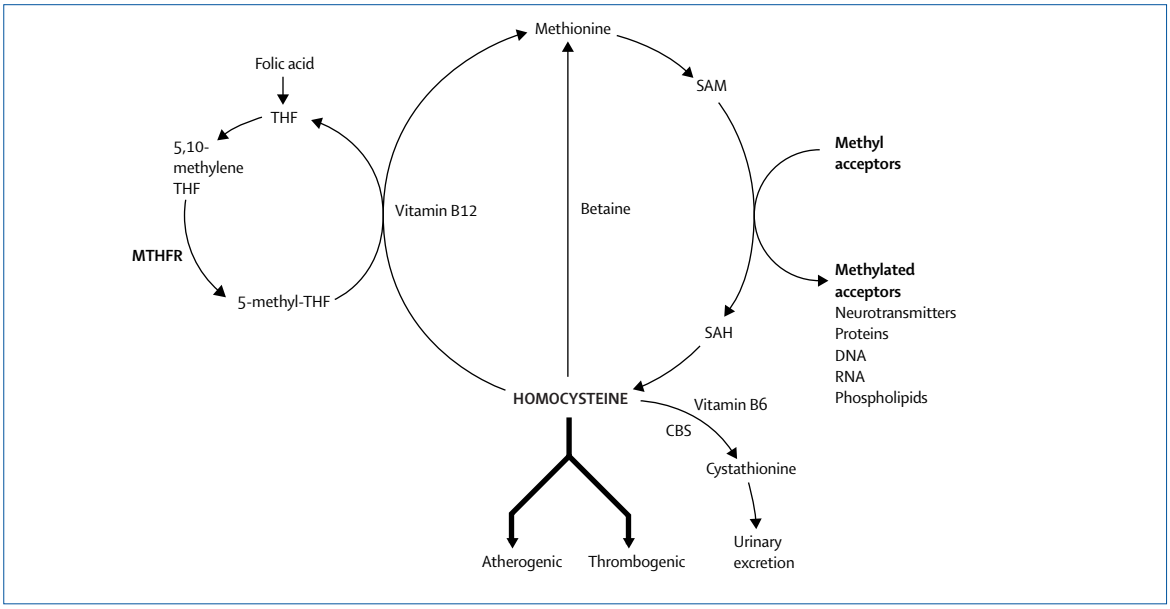
Systematic reviews of observational (cohort and case-control) studies have consistently shown a strong, positive, and dose-related association between the serum concentration of total homocysteine (tHcy) and the risk of stroke, which is independent of other vascular risk factors.[1,2] Laboratory studies have also shown that the association is biologically plausible.[3] However, causality has yet to be established because bias and confounding could not be eliminated in observational studies.[4] The stronger association reported in retrospective studies (in which researchers collected blood after stroke) than prospective studies, may reflect reverse causality bias (ie, acute stroke may increase tHcy).[1] Other known and unknown factors that increase both tHcy and stroke risk, (eg, smoking, lower socioeconomic class, existing atherosclerosis, and renal impairment) could have been confounding factors if they were not recorded in observational studies or adjusted for in the analyses. Deciding whether tHcy causes stroke is important because tHcy can be lowered effectively, safely, and affordably by folic acid, vitamin B12, and vitamin B6.[5,6]

The best method to minimise bias and confounding, and establish causality, is by randomisation. However, no randomised trials currently show that lowering tHcy reduces the frequency of stroke.[7,8] The Vitamins in Stroke Prevention (VISP) trial reported that lowering tHcy by 2 μmol/L (0·27 mg/L) with high-dose B-multivitamin therapy failed to prevent recurrent stroke in 3680 patients with recent ischaemic stroke.[7] The inability of homocysteine-lowering therapy to significantly reduce stroke in VISP, and prevent coronary in-stent restenosis in one small trial,[9] supports the hypothesis that elevated tHcy is a result of stroke and other vascular events, rather than the cause of them. However, VISP was statistically under-powered and could not exclude the possibility that B-multivitamin therapy reduced the relative risk of stroke by up to 20%.[8]

In today's *Lancet*, Juan Casas and colleagues present new evidence, on the basis of Mendelian randomisation, that an increased tHcy may be a causal risk factor for stroke. When homocysteine is re-methylated, the methyl donor (5-methyl tetrahydrofolate) comes from the metabolism of 5,10-methylene tetrahydrofolate by 5,10-methylene tetrahydrofolate reductase (MTHFR) (figure). About 10% of the population is homozygous for a 677 C→T polymorphism of the *MTHFR* gene. The TT polymorphism reduces production of the methyl donor 5-methyl tetrahydrofolate (akin to low dietary intake of folate) and increases tHcy by about 20% (ie about 2 μmol/L).[2,10] Consequently, the 677 C→T polymorphism produces a natural (Mendelian) randomisation. People are randomly allocated to groups with higher (TT) or lower tHcy (CC), because the polymorphism is distributed by random assortment of alleles during gamete production and fertilisation.[11,12] The groups should not differ systematically in any other way. For example, those with the TT genotype should be no more likely to be



Homocysteine metabolic pathways
In methylation pathway, homocysteine acquires methyl group from betaine, mainly in the liver, or from 5-methyl tetrahydrofolate, derived from metabolism of 5,10-methylene tetrahydrofolate by 5,10-methylene tetrahydrofolate reductase (MTHFR). CBS=cystathionine β synthase, SAH=S-adenosyl homocysteine, SAM=S-adenosyl methionine, THF=tetrahydrofolate. Reproduced with permission from the *Medical Journal of Australia* (*Med J Aust* 2004; **181:** 314–18).

smokers or of lower socioeconomic class than individuals with the CC genotype. However, because the effect of the *MTHFR* polymorphism on tHcy is modest, studies need huge numbers of patients to reliably identify or exclude a modest difference in stroke between people with and without the TT mutation.

In the largest meta-analysis to date of studies examining the association between MTHFR and stroke (111 studies), Casas and colleagues found that people who are homozygous (TT) for the *MTHFR* polymorphism have a significantly greater mean tHcy (weighted mean difference 1·93 μmol/L 95% CI 1·38–2·47), and risk of stroke (odds ratio 1·26, 1·14–1·40) than people who are homozygous and unaffected (CC). The greater risk of stroke conferred by *MTHFR*-TT is in proportion to the difference in tHcy that we can attribute to the polymorphism. Furthermore, the estimate of risk obtained from the meta-analysis of genetic association studies is similar to that obtained from meta-analyses of non-genetic observational studies.[1,2,10] Because the two types of studies have different sources of error, their consistency supports a causal role for tHcy. Also, the strength of the association between tHcy and stroke, estimated by Casas and colleagues from genetic association studies, is likely to be more accurate than the underestimates obtained by observational studies that correlated a single measurement of tHcy with stroke risk.

Yet, these results do not provide conclusive evidence of a causal association between tHcy and stroke. The *MTHFR* genotype might affect behavioural and socioeconomic factors by being associated with polymorphic variants of loci (linkage disequilibrium) that predispose people with the TT genotype to unhealthy behaviours, low socioeconomic status, or abnormal physiological risk factors for stroke (such as blood pressure or serum cholesterol), and confound the finding. Also, folate status and other environmental exposures that influence concentrations of tHcy, can modify or confound the causal association. The meta-analysis of Casas and colleagues might also be limited by publication and small-study bias, but study bias probably did not influence the results because the odds ratios are distributed symmetrically in relation to their standard deviations in the funnel plots.

Despite the potential limitations of Mendelian randomisation,[12] the triangular association between *MTHFR* genotype, tHcy, and stroke, described by Casas and colleagues, implies that higher concentrations of tHcy increase the risk of stroke, and that lowering tHcy by about 3 μmol/L with B vitamins should reduce the odds of stroke by about 20% in the overall population, independent of individual genotypes.[1,2] However, the relative increase in odds of stroke associated with the *MTHFR*-TT genotype and raised tHcy is small. Routine screening of *MTHFR* genotype would be an inefficient way to detect people at risk. Researchers must also discover if measuring tHcy can improve prediction of stroke on the basis of conventional risk factors.[13]

Even if it is established that tHcy increases the risk of stroke, it may not be wise for physicians to prescribe long-term B-vitamin therapy to a broad group. Not only does folate interact with some drugs, it can harm individuals with a deficiency of vitamin B12. Pteroylmonoglutamate (PGA), the folate used in vitamin supplements and food fortification, is not a natural co-enzyme.[14] With doses of PGA more than 400 μg (as are commonly taken), the absorption and biotransformation of PGA to methylfolate is saturated, and unmetabolised synthetic folate enters the bloodstream.[14] The biological effects, if any, of such long-term exposure are unknown. When the large randomised trials[15,16] are finished and a meta-analysis is done of the individual patients' data, physicians should know if it is safe and effective to prescribe long-term B-vitamin therapy to lower tHcy and stroke risk.

Graeme J Hankey, John W Eikelboom
Stroke Unit, Department of Neurology (GJH) and Department of Haematology, School of Medicine & Pharmacology (JWE), Royal Perth Hospital, Perth, Western Australia 6001, Australia
gjhankey@cyllene.uwa.edu.au

We are members of the steering committee of the Vitamins To Prevent Stroke (VITATOPS) trial.

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Effect of malaria on HIV-1 progression and transmission

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When two elephants collide beware, for the ground will shake. So it is with HIV-1 and malaria. Both infections are of such great public-health importance in tropical countries, particularly in sub-Saharan Africa, that any potential interaction should make us worry. In today's *Lancet,* James Kublin and colleagues report from a study in Malawi that acute malaria episodes are associated with transient increases of HIV-1 viral load in the blood, and they speculate that malaria episodes might accelerate HIV-1 disease progression and facilitate transmission.

Evidence is now emerging of the deleterious effects of HIV-1 infection on malaria. In pregnant women HIV-1 is associated with more peripheral and placental malaria, higher parasite densities, more fever, and increased risks of adverse birth outcomes.[1] Semi-immune non-pregnant adults infected by HIV-1 have higher rates of malaria infection and clinical disease,[2] while adults without malaria immunity have higher rates of severe malaria and death.[3]

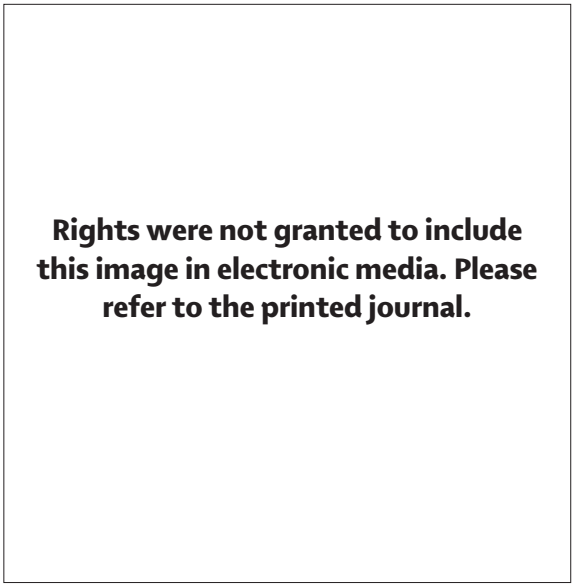
Less is known about the effects of malaria on HIV-1. Kublin and colleagues build on previous work from Malawi, which reported seven-fold higher HIV-1 viral loads in adults with acute malaria compared with those without, and that this increase resolved over a few weeks after antimalarial treatment.[4] By including a visit before the malaria episode as a reference, Kublin's study allows us to confidently blame the increase in viral load on the acute malaria episode.

The organisational difficulties of conducting field-based research of this nature in Africa should not be underestimated, and Kublin and colleagues must be congratulated for completing this careful and comprehensive study. However, because only 100 (35%) of the 287 participants who were followed up were included in the final analysis, we need to consider the possibility of selection bias. It is reassuring that the participants who were analysed were representative of patients with parasitaemia, and it seems logical that the increases in viral load tended to be greater with more severe episodes of malaria infection.

What are the clinical and public-health implications of Kublin's findings? Viral load is an important determinant of HIV-1 disease progression,[5] but the average viral-load increases reported in Kublin's study (0·25 log copies per mL) are unlikely to affect individual progression. Also, most of the increases were temporary, lasting less than 10 weeks, a short time in the context of an average disease course of 8–10 years. There is evidence that many opportunistic infections are associated with immune activation and higher viral loads,[6] but whether this relation is causative is debatable.[7,8] Researchers have associated many opportunistic infections with an increased risk of death,[7,9] and if we extrapolate from studies of the effects of viral loads on untreated survival times,[10] the modest and temporary increase in viral load reported by Kublin and colleagues would equate with a reduction of about 1 year in survival time. This reduction accords with reports of slightly shorter untreated median survival times from Africa, where the population has a higher burden of opportunistic infections than in developed countries.[11,12]

HIV-1 transmission is reported to double for each log rise in viral load.[13] Therefore the effects reported by Kublin and colleagues might equate with about a 50% increase in transmission during the short period of higher viral load in blood. The overall effect on transmission at the population level could usefully be studied further by computer modelling, taking into account individual variability in the period of higher viral load and the likelihood of reduced sexual activity during the acute malaria episode.

The greater effects of malaria reported in participants with well-preserved immune function is intriguing and needs further study. Intervention studies are also needed to further explain the relation between HIV and malaria, but these will be difficult to design and conduct in today's reality of antiretroviral therapy and cotrimoxazole prophylaxis, both of which might affect the attack rate and severity



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