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NUTRITION

AUGUST 2022

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Yuan S, Mason AM, Carter P, Burgess S, Larsson SC. Homocysteine, B vitamins, and cardiovascular disease: a Mendelian randomization study. BMC Medicine. 2021;19(1):97.

What We Know, Think We Know, or Are Starting to Know

Of the risk factors for chronic diseases, homocysteine has been a puzzling one. Homocysteine is an amino acid produced in the body from dietary intake of another amino acid, methionine, through what is known as the methylation cycle* (***See Geek Box**, below). When folate [vitamin B9], B6, or B12, are inadequate in the diet, homocysteine becomes elevated [1].

While there appears to be stronger supporting evidence for lowering homocysteine in relation to brain health [2–4], for cardiovascular health homocysteine has remained more of an enigma [5,6]. In epidemiological research, elevated homocysteine is a consistent independent risk factor for cardiovascular diseases [CVD], including stroke [7,8]. However, it remains unclear by what mechanisms homocysteine may influence CVD risk, although damage to the vascular system and increased arterial calcification have been implicated [5,9].

Another reason why there remains a lack of clarity on whether homocysteine is a cause or consequence of disease is the fact that, while vitamins B6/B9/B12 reliably lower homocysteine levels [10] there is little to no good evidence that this chain of B-vits>homocysteine lowers CVD risk [1].

Many of the potential reasons why the evidence from intervention trials of B-vitamins on CVD risk found 'null' results will be familiar to you from the previous Research Lecture on nutrient RCTs: participants with adequate levels of these vitamins at baseline, lack of sufficient contrast in intakes between treatment and placebo groups, and both groups also being on wider therapies, like statins and/or aspirin, which may have influenced the outcomes.

Mendelian randomisation [MR; *see Geek Box, below] provides another research design option which may allow for testing causal relationships independent of potential confounders, such as those identified in the large B-vitamin RCTs, above. The present study investigated the effects of genetic predisposition to higher blood levels of homocysteine, folate, B6, and B12, on cardiovascular disease.

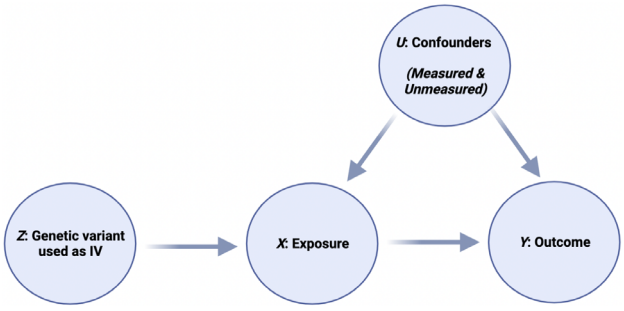


***Figure** illustrating the conceptual basis for MR. In this illustration, **Z** is the IV, a genetic variant associated with **X**, where **X** is the risk factor or "exposure". For example, **Z** could be a genetic variant which results in more LDL-receptors, which means that **X** would be low blood LDL-C levels. **Y** is the outcome, in this example, CVD. Thus, this graph is depicting the causal effect of **X** [low LDL-C] on **Y** [CVD]. Finally, **U** is any unmeasured confounder, i.e., "residual confounding". So, an MR study uses an IV [Z] to act as a proxy for an intervention of **X** on outcome **Y**.*

*Geek Box: Mendelian Randomisation

Mendelian randomisation [MR] is a principle of using genetics to mimic a long-term randomised controlled trial, particularly where a long-term intervention study may be unethical or practically infeasible. Because an individuals' genes are 'assigned' when they are conceived, this in effect it is the purest form of randomisation, i.e., the genetic lottery from Mom and Pops.

Well-conducted MR can provide an unconfounded estimate of the relationship between an exposure and an outcome. It is unconfounded because the genetic variant results in a certain physiological response that is independent of other considerations. Thus, to be properly conducted, a MR study has to satisfy three criteria:

1. *The genetic variant must be associated with the specific mediating exposure', e.g., LDL-C;*
2. *The genetic variant must not be associated with any potential confounders that could influence the outcome, and;*
3. *The genetic variant must only influence the disease outcome through the specific exposure pathway, not through other mechanisms.*

An IV is only valid where the 3 assumptions above hold. This is crucial, because it means that claims of "causality" can only be made where these assumptions are met. Where there may be factors that undermine these assumptions, then an MR study should be considered genetic associations, not necessarily a cause-effect relationship. Several potential issues may arise for nutrition research.

In the first instance, examples of where a genetic variant provides a strong IV for mimicking dietary intake are rare. And importantly, a genetic variant IV may only be associated with a specific, isolated tissue compartment, e.g., plasma, which may not be the sole pathway through which a given nutrient may be associated with an outcome. Where an IV mimics nutrient levels in a specific tissue compartment, conclusions should be confined to that tissue compartment, not stated as the effect of a nutrient broadly in relation to the outcome of interest.

When long-term randomised studies are not possible, Mendelian randomisation is a powerful tool to examine potential cause-effect relationships. But we should temper our enthusiasm for thinking anything genetic solves all methodological challenges in our field, as MR of nutritional exposures faces several methodological challenges itself that need to be considered.

The Study

The study identified genetic variants associated with 12 different CVD endpoints from large genetic databases, including the UK Biobank and Finnish FinnGen cohorts, which served as the outcome data.

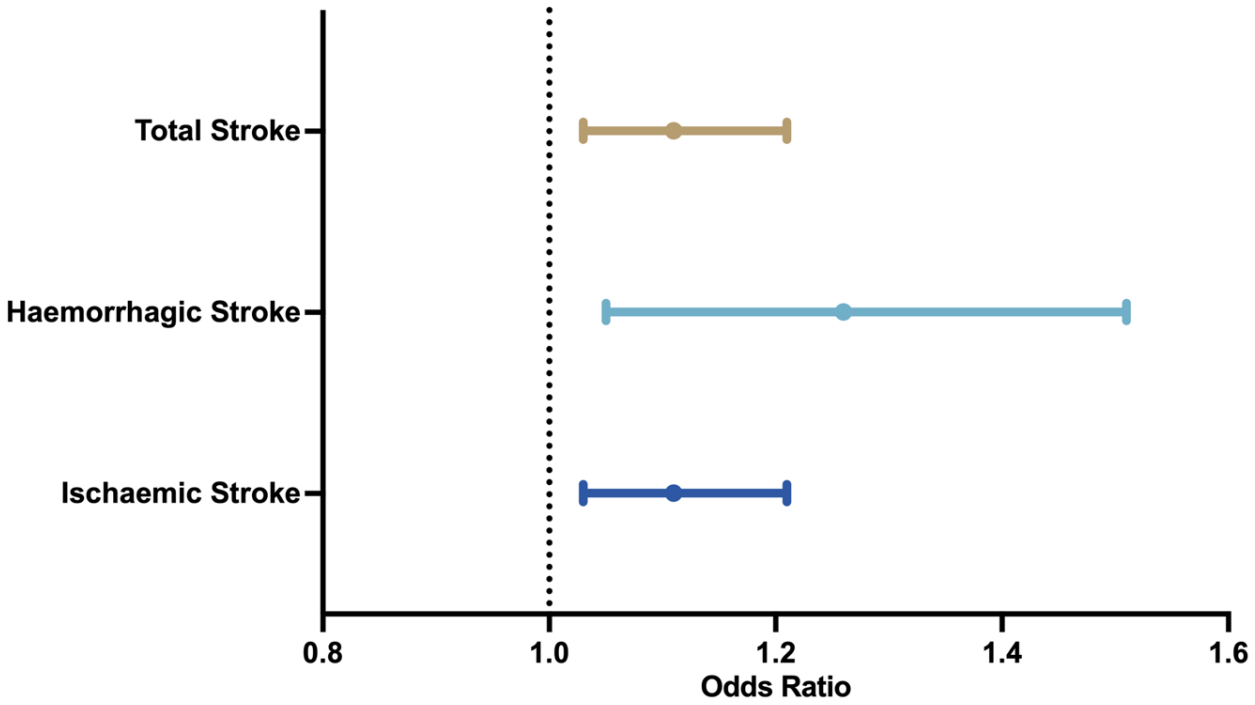
For the exposures, the study utilised genome-wide association studies on:

- Homocysteine [data from *n* = 44,147 individuals]
- Vitamin B9 folate (data from *n* = 37,465 individuals]
- Vitamin B6 (data from *n* = 1,864 individuals]
- Vitamin B12 (data from *n* = 45,576 individuals]

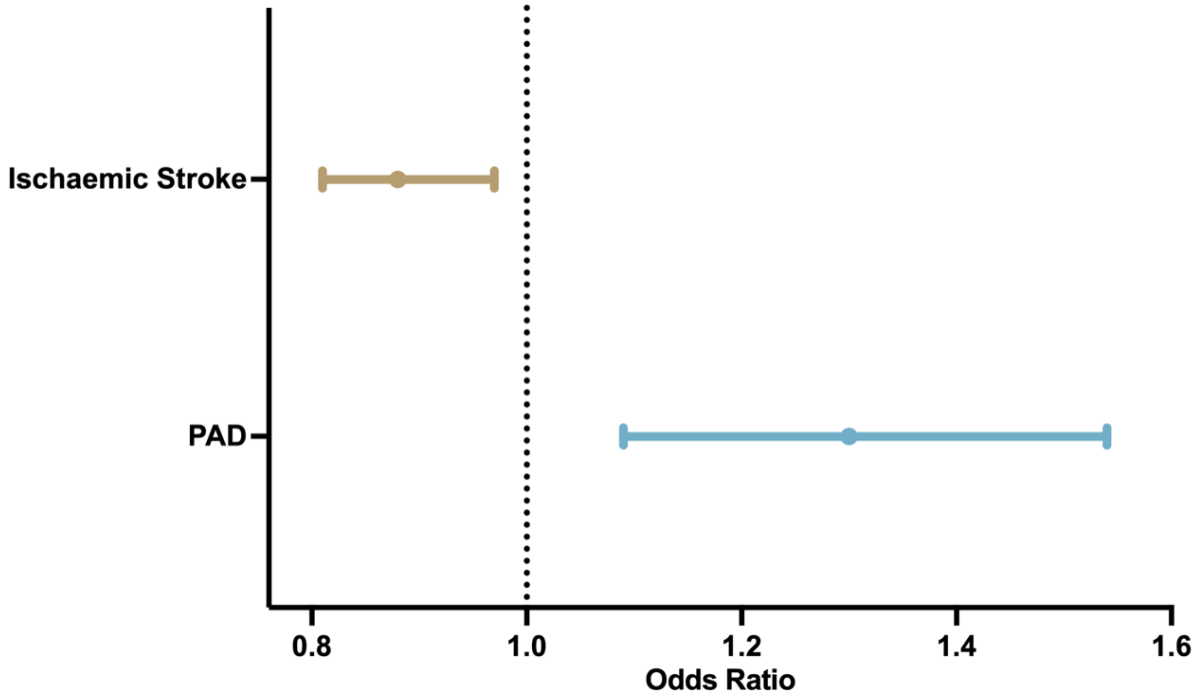
The analysis then tested the associations between genetically predicted levels of homocysteine [HCY], B6, B9, and B12, and CVD endpoints. The outcomes were reported as odds ratios [OR] with 95% confidence intervals [CI].

Results: There were no significant genetic associations identified between vitamin B12 and any CVD outcome. Thus, the findings detailed below are for the other outcomes.

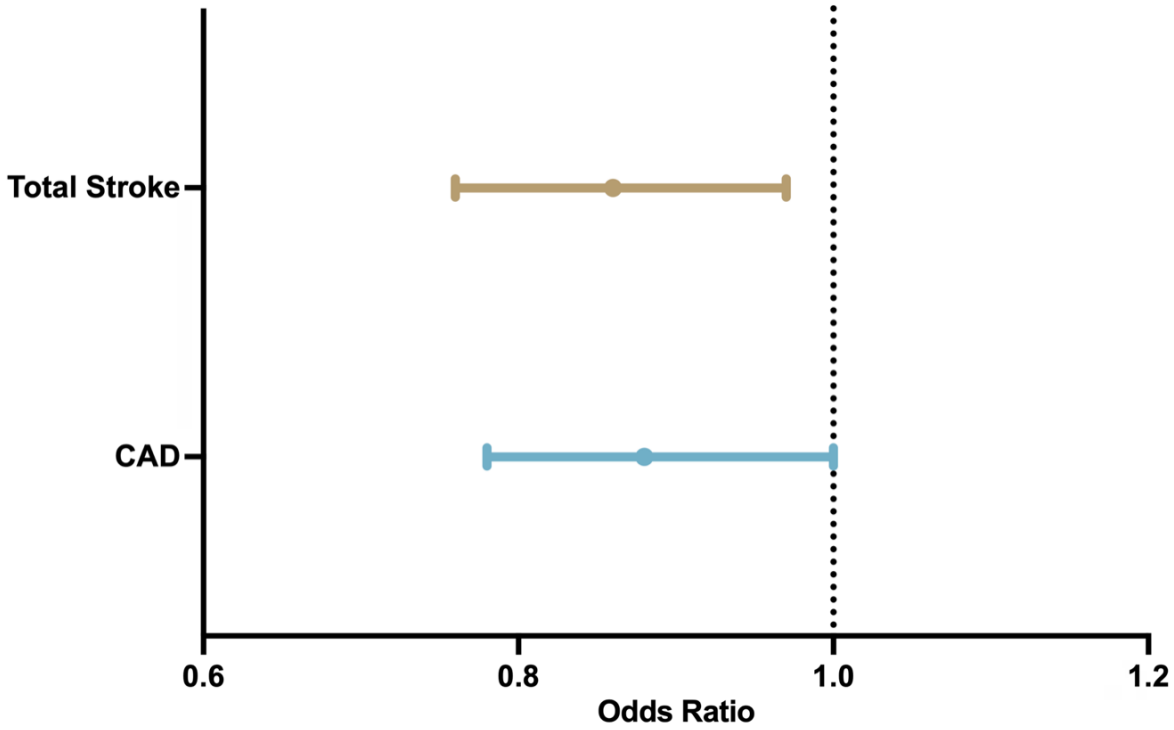
- ***Homocysteine and Stroke*:** For genetically higher HCY levels there was 11% higher odds of total stroke [OR 1.11, 95% CI 1.03 to 1.21], a 26% [OR 1.26, 95% CI 1.05 to 1.51] higher odds for subarachnoid haemorrhage, and an 11% [OR 1.11, 95% CI 1.03 to 1.21] higher odds for ischaemic stroke. There was no significant association between genetically higher HCY levels and other CVD endpoints.



- ***Vitamin B6*:** For genetically higher B6 levels there was 12% lower odds of ischaemic stroke [OR 0.88, 95% CI 0.81 to 0.97], and 30% higher odds for peripheral artery disease [OR 1.30, 95% CI 1.09 to 1.54].



- ***Vitamin B9 [Folate]*:** For genetically higher B9 levels there was 12% lower odds of coronary artery disease [CAD; OR 0.88, 95% CI 0.78 to 1.00], and 14% lower odds for total stroke [OR 0.86, 95% CI 0.76 to 0.97].



The Critical Breakdown

Pros: A total of 8 different genetic studies were utilised to determine genetic associations with CVD endpoints. This allowed for pooling of cases from different genetic databases to increase the robustness of the associations between genetic variants and CVD risk. The statistical analysis included several methods specific to MR that allow for the identification of outliers and genetic variants that may influence outcomes through other mechanisms [known as pleiotropy].

Cons: The genetic variants identified for the B-vitamins were only associated with a fraction of the variance in levels of B-vitamins, 1% for folate, 1.3% for B6, and 6% for B12. Again, this is an important limitation for MR studies of nutritional exposures; you can' t look for strong genetic associations if genetics don' t strongly influence nutritional status. Because much of the influence of levels of a nutrient in the body may not be genetic, and more related to actual dietary [including supplemental] intakes, itself influenced by behaviours and the environment. There were low numbers of cases for the CVD endpoints in the analysis of specific vitamins, in particular vitamin B6, which could weaken the power of the study to detect associations. The genetic variants for vitamin B6 have not been validated. Finally, the genetic databases were confined to populations of European ancestry, and may not be generalisable to other population groups.

Key Characteristic

One of the ongoing questions regarding biomarkers of CVD risk, as we discussed under the ***What We Know*** section, above, has been the utility of HCY as a marker. Given that the present study used genetics to investigate the influence of genetically higher HCY on CVD risk, is this any more reliable as a finding?

The open questions mostly have been in relation to mildly and moderately elevated HCY of ranges of >15µmol/L and >30µmol/L, respectively. One aspect to this question over mild-moderate elevations in HCY is the fact that HCY is associated with other risk factors for CVD, in particular increased blood pressure and kidney function [(11)]. A previous meta-analysis of prospective studies on HCY found the each 5µmol/L increase in HCY was associated with 20% increased odds for coronary heart disease [OR 1.20, 95% CI 1.14 to 1.25] [(12)].

The associations appear to be strongest for vascular diseases such as stroke, with a 59% [OR 1.59, 95% CI 1.29 to 1.96] higher odds in prospective studies [(13)]. This relationship with stroke has been confirmed in other MR genetic studies [(14)]. With the present study now added, do we deem moderately elevated HCY to be a causal risk factor for stroke? For other CVD' s, it remains arguable that – like TMAO – elevated HCY is a marker that is secondary to other disease.

However, for vascular diseases, the strength of evidence now suggests that elevated HCY is an antecedent to vascular disease that causally increases risk of stroke. That the present study adjusted for the effects of the selected genetic variants that also influence blood pressure or kidney disease strengthens that causal inference.

Interesting Finding

Hands down for us with our nutrition hats on, the most interesting finding in this study is for vitamin B9 [folate]. In this study, genetically predicted higher folate levels were associated with significant reductions in both total stroke and CAD. And we know that folic acid supplementation will reduce HCY [more under ***Relevance***, below], but does that translate into lower CVD risk?

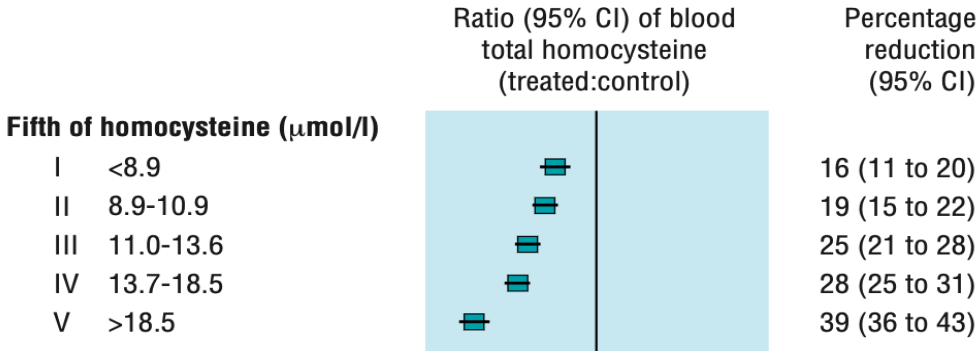
This is where the evidence has not been clear cut. One factor that we have discussed at length for nutrient RCTs is the importance of baseline nutrient status in interventions. As dietary folate intake is commonly low in populations without food fortification policies, one way to tease out any potential effect of folic acid supplementation is by considering fortification.

In a meta-analysis that took this approach, folic acid supplementation was shown to significantly lower stroke risk by 12%, but in studies conducted in countries *without* folate food fortification policies; there is no association from studies in countries that had existing folate fortification policies [15]. The reduction in HCY levels from folic acid supplementation was also significantly different based on folate fortification status, with decreases of 26% and 18% in regions without and without folate fortification, respectively [15].

Relevance

To start with, we can hang our hat on the evidence that vitamin B9, as folic acid, reliably lower HCY levels, and that this effect linearly increases relative to baseline HCY levels [10]. The **figure** below is from the Homocysteine Lowering Trialists' Collaboration [10], and illustrates the effect of folic acid supplementation on HCY levels. As you can see in the far-left column, HCY levels were stratified into quintiles; and on the far-right column you can see the percentage reduction [and 95% CI] in HCY levels.

You can see that this ranges from a 16% [11% to 20%] reduction in HCY levels from folic acid supplementation in people with baseline HCY of <8.9µmol/L, up to a 39% [36% to 43%] reduction in people with baseline HCY of >18.5µmol/L.



And as highlighted under Interesting Finding, above, the importance of baseline nutrient status is critical to determining any effect of folic acid supplementation on actual CVD endpoints, in particular stroke. What can we say of this relationship? In the most recent meta-analysis of RCTs, folic acid supplementation lowered stroke risk by 12% [RR 0.88, 95% CI 0.80 to 0.98]. But guess what? That effect was most pronounced in participants with low baseline folate levels; a 21% relative risk reduction [RR 0.79, 95% CI 0.69 to 0.89] with more precise and robust confidence intervals [16].

Application to Practice

Although this analysis found no associations with B12, we know that it is crucial to maintain nutrition adequacy of B12, especially in those not consuming animal produce. And the B6 findings from this should be treated as exploratory only.

Yet while there remain limits to MR for nutritional exposures, what makes the present analysis slightly more robust than others is how much wider literature is available on the genetics of folate metabolism and HCY, in particular the MTHFR enzyme and methylation cycle.

If we parse the totality of evidence for folic acid supplementation and CVD, and factor in those common nutrition-specific methodological challenges for RCTs, a more consistent picture of risk reduction emerges that is, surprise-surprise, most apparent in individuals with low folate intake. This is another line of evidence that supports prophylactic supplementation of folic acid in people who don't eat enough greens [i.e., most of the population] and/or women in reproductive life stages.

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