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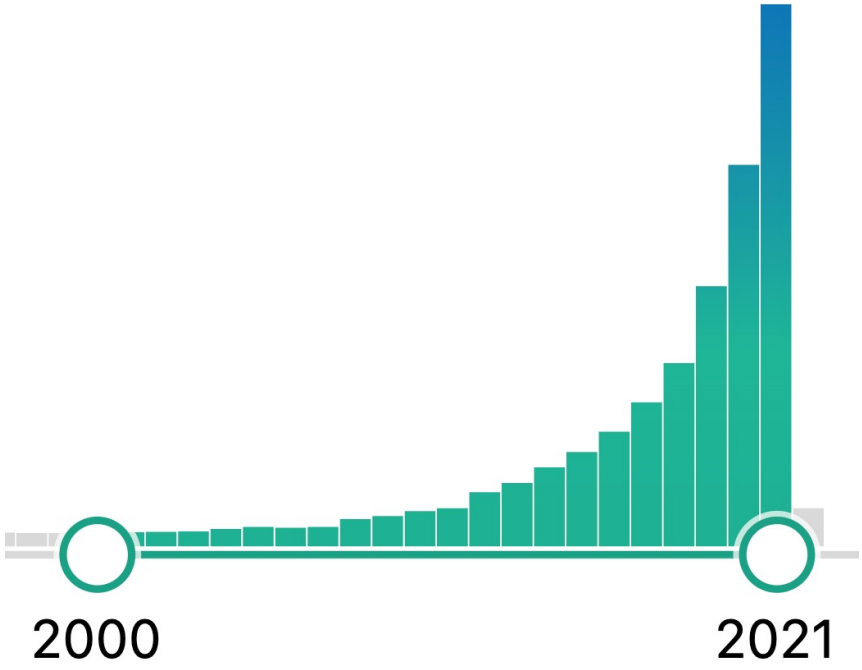
Wang T, Xu L. Circulating Vitamin E Levels and Risk of Coronary Artery Disease and Myocardial Infarction: A Mendelian Randomization Study. Nutrients. 2019;11(9):2153.

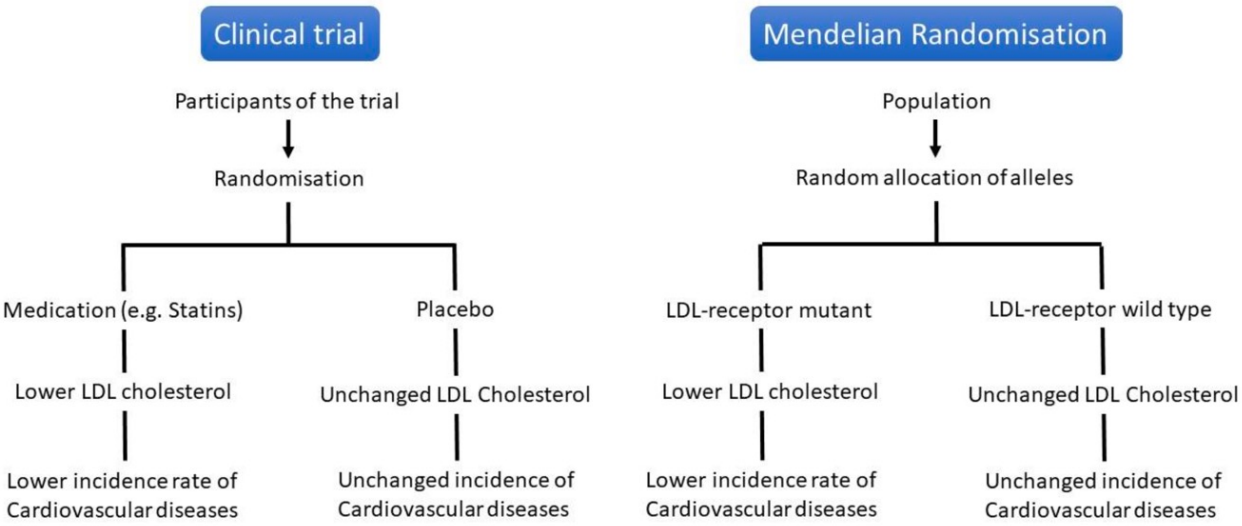
What We Know, Think We Know, or Are Starting to Know

It's about time we started getting some Mendelian Randomisation [MR] studies into the Deepdive rotation, given this research methodology seems to be bringing sexy back (Timberlake et al., 2006). But seriously, look at the exponential growth of MR studies in the PubMed database over the last 4yrs:



Today's Deepdive is going to focus on getting you an understanding for what MR is, what assumptions underpin this research methodology, and what utility it may serve for nutrition research. We'll do this through the prism of a recent 2019 study on vitamin E and heart disease.

The MR method is named after Gregor Mendel, the father of modern genetics who discovered the principle of genetic inheritance. While that explains the 'Mendelian' part, you may be thinking, *"why 'randomisation'"?* Because the genes you inherited from Mum and Dad were *"assigned"* to you *randomly*. If you've siblings, for example, you might wonder why you got Dad's height and they got Mum's eyes. This can then be applied to research, as the following illustration shows:



*The **Figure** above illustrates the difference between a randomised controlled clinical trial [**left**] and an MR study [**right**], using the example of cardiovascular disease [CVD].*

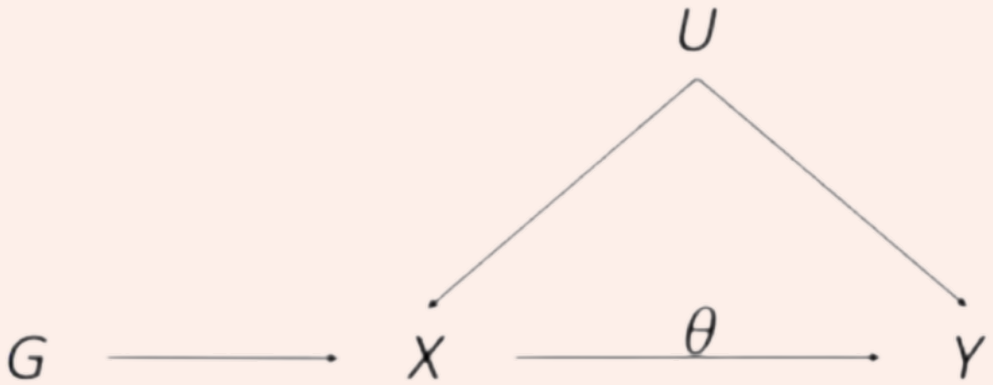
In the RCT, participants are randomly assigned to the intervention to lower LDL-C [e.g., statins] or placebo, which would leave LDL-C unchanged. In MR, people in the population would have been randomly allocated a version of a gene, known as an 'allele'. An allele is one version of a gene which has two or more versions of it. People inherit two alleles for every gene, one from each parent.

In this example, people in the population have been 'assigned' a mutated version of a gene which leads to greater expression of the LDL-receptor, so they clear more cholesterol from the blood and have lower rates of heart disease. And because they were born with this allele, this means they have lifelong lower LDL-C levels, which means that researchers can consider this as an "exposure" which is not confounded by other factors, e.g., diet. In this context, an MR study can be used to mimic an RCT, because the researchers can compare an "exposure group" of people with genetically lower LDL-C to a "control group" of people with 'wild type' genes, which is the normal gene without any mutations.

An MR study is, in principle, a halfway house between observational epidemiology and an RCT. Because genes are randomly assigned at birth, it is considered that exposure-outcome findings are free from what is known in epidemiology as "residual confounding", which means that an outcome is potentially confounded by some unknown factors that the researchers did not measure, or perhaps that we don't even know about yet. This is why MR studies are considered to show "causal" relationships between an exposure and outcome. However for an MR study to be considered causal, it must meet certain assumptions: those are discussed in more detail in the **Geek Box***, below. Let's get to the study…

*Geek Box: Assumptions of Mendelian Randomisation

All MR studies start with a genetic variant, which for an MR is known as an "instrumental variable", or IV. The best way to illustrate these concepts is with the use of 'directed acyclic graphs', or DAG. DAG are graphs which illustrate the direction of relationships and are useful to illustrate causal concepts. Here is a DAG for MR:



*In this illustration, **G** is the IV, a genetic variant associated with **X**, where **X** is the risk factor or "exposure". For example, **G** 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.* θ *in this graph is 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 [**G**] to act as a proxy for, e.g., an intervention lowering LDL-C.*

Now, about those assumptions…

*For an IV [**G** in our graph above] to be valid, it must meet three assumptions:*

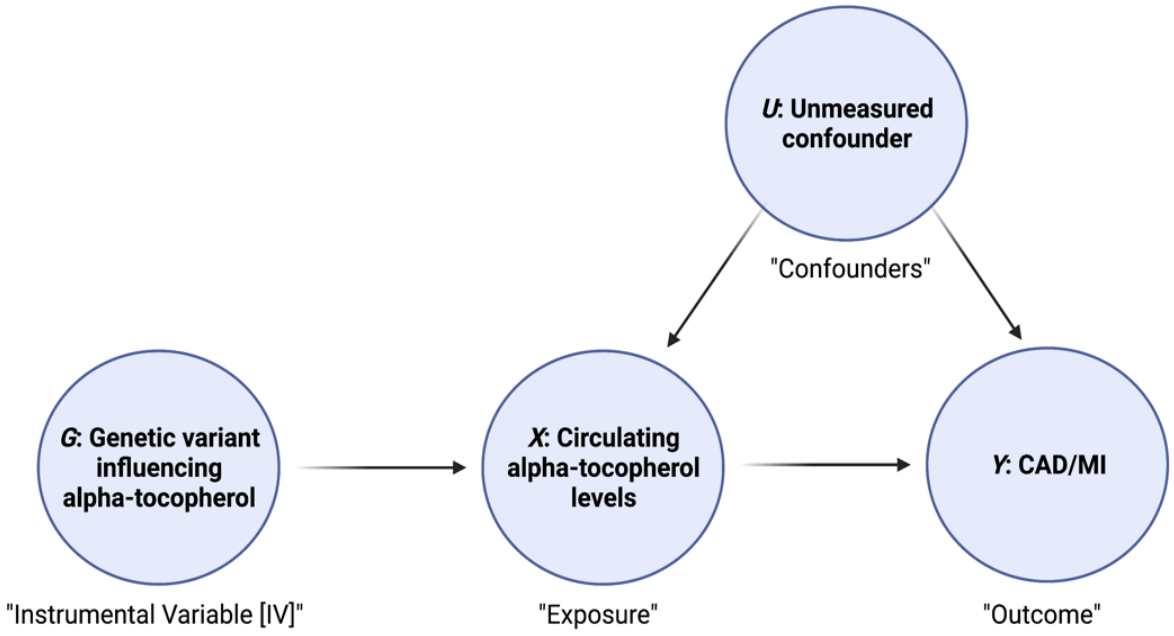
1. ***Relevance:** The genetic variant, **G**, is associated with the risk factor, **X***
2. ***Exchangeability**: The variant is independent of confounders, **U***
3. ***Exclusion-restriction:** The variant has no effect on the outcome, **Y**. The outcome, **Y** should be conditional on the effect of the risk factor, **X** (and potential confounders, **U**, but the IV is independent of those confounders)*

*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 hold. However, in practice there is no easy way of testing that all assumptions hold, unlike in other statistical methods where the assumptions can be tested [e.g., the assumption that data follows a normal distributed can be tested with both statistical tests and by visual inspecting normality graphs].*

*In reality, the assumptions are addressed by considering different factors, e.g., the biological plausibility of the IV [i.e., is it biologically plausible that a gene that effects the LDL-receptor would lower LDL-C levels], by examining whether the estimates of the effect of the risk factor **X** on the outcome **Y** are similar across different analyses [i.e., does low LDL-C from different LDL-receptor variants have similar effects on CVD], and by considering whether the effects are not modified by other factors. We expand more on the most important assumption, that of Exclusion-Restriction, under **Key Characteristic**, below.*

The Study

The present study was an MR analysis of the relationship between circulating alpha-tocopherol levels and coronary artery disease [CAD], myocardial infarction [MI], and type-2 diabetes [T2D]. Using genetic data from 7,781 individuals from three different studies, the researchers identified three genetic variants associated with circulating levels of alpha-tocopherol. Genetic associations between the risk factors LDL, HDL, and triglycerides, and CAD/MI were also extracted from genetic databases. The graph below illustrates the study design:



Results: The study calculated odds ratios [OR] and 95% confidence intervals [CI] for each genetically predicted 1mg/L increase in alpha-tocopherol [AT] levels.

- ***CAD/MI*:** A 1mg/L increase in AT was associated with a 5% higher [OR 1.05, 95% CI 1.03 – 1.05] CAD risk, and 4% higher [OR 1.04, 95% CI 1.03 – 1.05] for MI.
- ***Blood Lipids*:** A 1mg/L increase in AT was associated with a higher LDL-C levels, triglycerides, total cholesterol, and lower HDL-C levels.
- ***T2D*:** There was no association between genetically predicted circulating AT levels and T2D or fasting blood glucose levels.

The Critical Breakdown

Pros: The main strength of the present study is the use of wide genetic databases to determine genes associated with circulating AT levels, and genetic outcomes in relation to risk factors. The first assumption of MR appear to be met in relation to the study, i.e., the genes were associated with the circulating levels of AT.

Cons: Although the statistical tests of the association between the IV and the risk factor, i.e., the gene and circulating AT levels, were very strong, the study runs into trouble on the other assumptions [which we go into in more detail in the following sections]. Indeed, one limitation is that the authors give little to no discussion in their methods to the assumptions for their selection IV, and any good MR paper should really elaborate on how these assumptions may be met. To the authors' credit, they do highlight themselves that the analysis focused on AT specifically, and although they refer to "vitamin E", AT is one of eight isoforms of vitamin E and other isoforms, gamma-tocopherol in particular, may also be relevant for health effects of this nutrient.

Key Characteristic

Recall the assumptions of MR set out in the **Geek Box**, above? It's crucial that we grasp that claims of causality can *only* be made when these assumptions are met; when a given MR study does not appear to meet these assumptions, then the findings are *genetic associations*, i.e., are correlational results similar to the findings from a traditional epidemiological study. The most important assumption is No.3: **Exclusion-Restriction**. This is because a genetic variant is supposed to serve as a proxy exposure associated with an outcome. Another way of phrasing this assumption is that the IV is related to the outcome *only through* the exposure/risk factor. For nutrition, this is where things get tricky.

So far, we have used the example of LDL-C, but this is easier to satisfy the assumptions of MR because, with prior knowledge, we know that *every* genetic variant that *increases* blood LDL-C levels, like in Familial Hypercholesterolemia [FH], cause defects in the LDL-receptor that stop LDL-C being cleared. We know that *every* genetic variant that *decreases* LDL-C does the *opposite*: increases LDL-receptor activity and therefore clearance for LDL-C from the blood. We know that *every* drug that lowers CVD risk does so by lowering LDL-C *through increasing LDL-receptor activity*. I'm hammering home the point that *all roads go through the LDL-receptor*, and only that final pathway. Thus, we can be certain that a genetic variant which influences LDL-C levels has no other way of affecting the outcome: ***Y*** is conditional on the effect of the risk factor, ***X***, and the assumption of Exclusion-Restriction is satisfied.

But for nutrition…well, that just is not how nutrients act. Nutrients are *polyvalent*, meaning they act in multiple tissues, through multiple pathways. This is particularly the case for a complex nutrient like vitamin E, where there may be roles for other tocopherols in anti-inflammatory and anti-oxidative processes [(1)]. The main point that I want to stress here is that, where an IV of putative nutrient exposure mimics levels of a nutrient *in a specific tissue compartment*, then the conclusions *should be confined to that specific tissue compartment*, rather that claimed to represent the broad effects of that nutrient on the outcome of interest.

Interesting Finding

The interesting finding of this study is also the finding that serves as the amber light for thinking about MR and nutrition: that higher vitamin was associated with higher LDL-C. Eh, are we sure about this direction of effect? The finding stated that each 1mg/L *increase* in vitamin E was associated with elevated LDL-C levels, implying that the direction of effect was that LDL-C follows the increase in vitamin E.

This just doesn't really hold with prior knowledge. The primary carrier of vitamin E, primarily AT [although some other tocopherols are in the mix], is LDL. Each LDL particle carries between 5 to 9 vitamin E molecules [(2)]. And, drumroll please…guess what is the primary causal risk factor for CVD, including CAD? Yeah, that would be LDL [(3,4)].

The present study assumed that any potential confounding would only be through the effect of vitamin E on blood lipids [i.e., IV>LDL>CAD], but the well-established causal role of LDL-C in heart disease means the association between higher circulating vitamin E may in fact be a proxy for higher circulating LDL. The fact that higher LDL was associated with higher vitamin E is not surprising given that LDL is the primary carrier of vitamin E in the circulation.

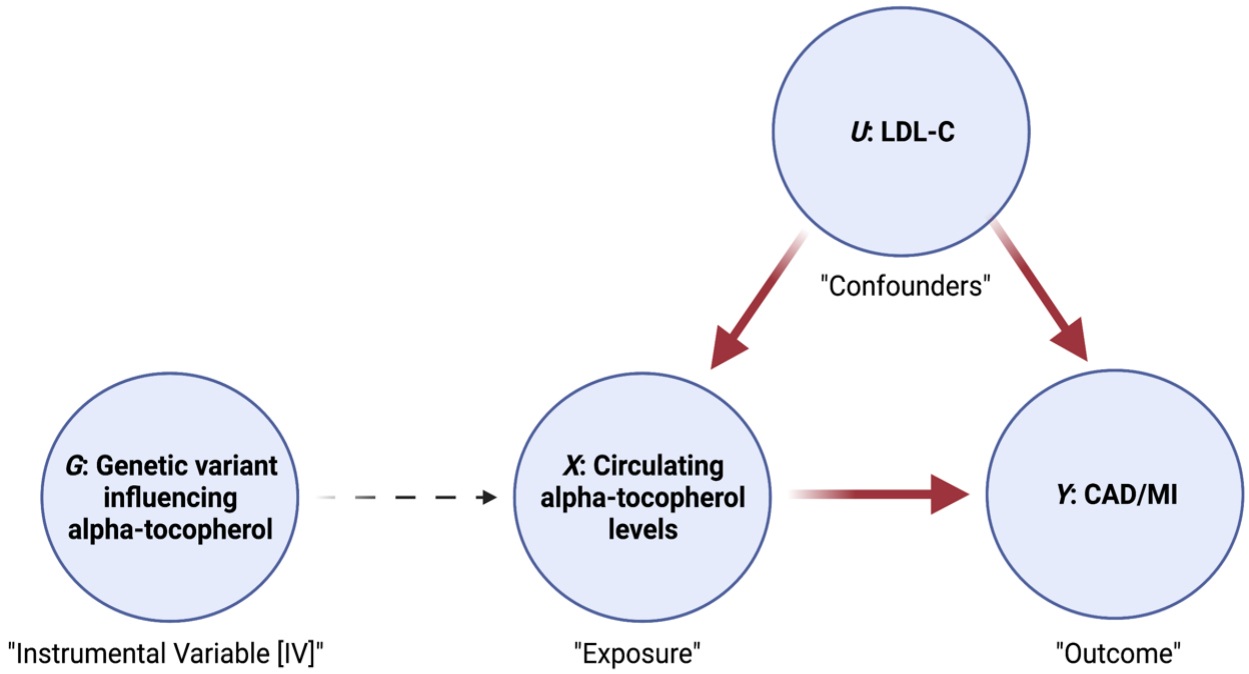


Figure *illustrating that, although Assumption No.1 was satisfied in this study and the genetic variants were associated with circulating vitamin E levels, the fact that these levels correlated with high LDL means the potential for this to be the operative causal factor, given what we know about LDL, is too strong to rule out.*

Relevance

I chose this paper in particular because it is becoming for MR to be treated like it is bigger than Prince. Let's be clear: *nothing* is bigger than Prince. Because of the epistemic conflict between the biomedical model and nutrition science, there is an enthusiasm and idealistic hope that MR is going to come to the rescue of the field and solve all of the apparent problems, specifically a reliance on epidemiology.

However, recall this crucial point: where an MR does not meet the assumptions for a valid IV, then the findings are *genetic associations*, not causal relationships. An 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. As a result, the reality for nutrition is that, to date, cases where a genetic variant provides a strong IV for mimicking dietary intake are rare [5].

MR is often posited to act as the 'settler' if there is discord between epidemiological findings and RCTs. Indeed, that was the basis of the present study in relation to vitamin E. But this may also be a flawed premise, because it assumes that if there is discord, that it reflects two genuinely competing findings, rather than findings that have not been properly reconciled.

For example, epidemiological associations between vitamin E and protective effects against CVD were seemingly contradicted by large intervention studies that subsequently generated 'null' findings [6]. In the Heart Outcomes Prevention Evaluation [HOPE] intervention using 400IU supplemental α-tocopherol, there was no significant difference in any outcome between groups, yielding the conclusion "no effect of vitamin E" [7]. However, both the intervention group and placebo group had adequate levels of plasma vitamin E. In HOPE, there was also some suggestion of *increased* risk of heart failure in the supplement group. But this highlights the difference between a dietary exposure consumed in the context of foods and a whole dietary pattern compared to high-dose supplementation and the biphasic dose-responses which defines antioxidant activity, with higher levels potentially associated with adverse outcomes [8,9].

The present MR study purports to uphold the latter findings, i.e., the findings in RCTs of potential *higher* risk associated with vitamin E. But this is premature; it could only deduce that conclusion if the assumptions for the IV for vitamin E were all met, and as we have seen, this is difficult to sustain.

MR is an exciting research design and holds promise for nutrition research in providing a link in the chain for causal inference of nutritional exposures [5]. However, while MR certainly has potential for a highly valuable additional line of inquiry for nutrition science, it is not an assumption-free design, and the characteristics of nutrient action must be considered in relation to establishing the assumptions of validity for IV's, and in the context of prior knowledge.

Application to Practice

The authors of the present study conclude that the "*present MR analysis showed that higher vitamin E was causally associated with high risks of CAD and MI.*" Causal? No, this is a genetic association, one with the potential major confounder of LDL-C. To quote a recent paper by our Finnish friend Pauli Ohukainen, beware *"the perils of hasty causal expectations"* [5]. I wouldn' t stop eating vitamin E-rich foods just yet.

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