Challenges in Interpreting Multivariable Mendelian Randomization: Might "Good Cholesterol" Be Good After All?

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Understanding the causal basis of disease is important so that we approach disease prevention and treatment using a valid etiologic framework. Blood lipids play an important role in the shuttling of nutrients (in the form of triglycerides and fatty acids) and cholesterol from the diet

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to the peripheral tissues. Certain types of blood lipids (eg, low-density lipoprotein cholesterol [LDL-C] and probably triglycerides [TG]) are atherogenic and lead to higher risks for coronary heart disease (CHD).[1-6] The role of high-density lipoprotein cholesterol (HDL-C) to date has been more elusive.[2] Its central purported role is the reverse transport of cholesterol, which theoretically should lead to a net reduction in atheroma in the tunica intima of the arterial vasculature (with supposedly commensurate reductions in risk for vascular disease). This idea has come under scrutiny in recent years in response to accumulating scientific evidence[5,7-9] suggesting that increases in conventional measures of HDL-C may not lead to tangible benefits to CHD. However, this does not rule out a potentially important role of HDL-C in other diseases (including other vascular diseases, such as abdominal aortic aneurysm).[10-12]

In the accompanying study by Lanktree et al,[10] the authors aimed to dissect the nature of the relationship between blood lipid concentrations and chronic kidney disease (CKD). Although traditional observational data provide evidence that HDL-C concentration is inversely associated with risk of kidney disease,[13] such findings need to be interpreted with caution because the inherent limitations of observational research (namely, confounding and reverse causality) can distort findings. For example, the inverse association of HDL-C concentration with CHD seen in conventional observational studies[2] has not been validated in clinical trials[14,15]; similar confounding could be at play in the reported associations of HDL-C with other diseases, including CKD. Mendelian randomization (MR) is an alternative analytical approach that uses genetic variants that are inherited at random and are non-modifiable to make causal inference that should be relatively free of confounding and reverse causality.[16,17] However, just as traditional observational studies have their inherent limitations, MR studies also make assumptions and have potential limitations that can cloud their interpretation.[18-21] Recent methodological developments in MR have allowed a relaxation of some of these assumptions and provide sensitivity analyses with which to scrutinize estimates from MR in further detail.[18,22-25] Improved methodologies, together with the availability of data platforms such as MR-Base[26] and its extensions implemented in "MR of everything vs everything"[27] facilitate the conduct of MR analyses for multiple exposures and multiple outcomes.

Lanktree et al[10] used genetic variants identified from genome-wide association analyses that are associated with different concentrations of the 3 main blood lipid fractions (namely LDL-C, HDL-C, and TG) reported by the Global Lipids Genetics Consortium (GLGC).[28] These genetic variants were used to gauge insight into the causal relationships of blood lipids with 3 markers of kidney function reported from genome-wide association studies by the CKD Genetics (CKDGen) consortium[29]: (1) estimated glomerular filtration rate (eGFR) as a continuous trait (percent difference), (2) dichotomized eGFR (odds ratio of eGFR < 60 mL/min/1.73 m^2), and (3) albumin-creatinine ratio (ACR; percent difference). Using a 2-sample MR framework (in which the single-nucleotide polymorphism [SNP]-to-exposure and SNP-to-outcome estimates were obtained from predominantly nonoverlapping data sets, with the authors reporting that <10% of data overlapped between GLGC and CKDGen), Lanktree et al[10] provide evidence in support of blood lipid concentrations being linked to kidney function.

The authors identified that genetically elevated HDL-C concentrations were associated with better eGFRs (a higher percent difference in eGFR and lower risk for eGFR < 60 mL/min/1.73 m^2) and lower ACR using genetic instruments for HDL-C. These associations remained robust to adjustment for the association of the genetic variants with LDL-C, TG, and hemoglobin A_{1c} concentrations and blood pressure in so-called multivariable MR.[25] Such findings are commensurate with HDL-C having a potentially protective role in kidney function. However, the authors note that treatment trials of drugs that increase HDL-C concentrations (such as the Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes [AIM-HIGH][30] trial of niacin) had no discernible effect on kidney function. This discrepancy between a genetic instrument for HDL-C versus a specific therapy may arise for various reasons. First, a trial of an individual drug (such as niacin) may have a separate effect on kidney function compared with that of the overall causal effect of HDL-C concentration, the latter being a broad biomarker that has a genetic architecture comprising multiple independent loci.[28]

Second, blood lipids may play a role in the cause of kidney disease only at certain periods of life and not at others, a so-called "critical period" effect.[19] As an extension to the critical period effect, lipids could be a causal risk factor for kidney disease progression rather than disease onset; disease initiation and progression could have distinct causes, meaning that exposures causal for disease onset may not be necessarily causal for progression (and vice versa).[31]

For LDL-C–related SNPs, a relationship of genetically elevated LDL-C concentration with a higher percentage difference in eGFR (ie, better kidney function) was identified when LDL-C SNPs were examined on their own. However, incorporating the relationship of the LDL-C SNPs with other lipids, hemoglobin A_{1c}, and blood pressure, the association between genetically elevated LDL-C concentration with percentage difference in eGFR became less pronounced. For TG, the relationship of genetically elevated TG concentration with percentage difference in eGFR, while weak on its individual analysis, became more pronounced on adjustment for these other traits.

These relationships are nontrivial to tease apart. For example, adjusting the LDL-C SNPs for hemoglobin A_{1c} concentration (a marker of dysglycemia) could adjust for a potential mediating effect of diabetes on CKD, thereby resulting in the attenuation of the relationship between LDL-C concentration and percentage difference in eGFR that the authors report. Prior MR studies have shown that higher LDL-C concentration is related to lower risk for type 2 diabetes mellitus,[8,32] meaning that a causal pathway could exist from higher LDL-C concentration to lower risk for type 2 diabetes mellitus and lower risk for CKD. Alternatively, the wider 95% confidence intervals and resultant attenuated effect on percentage difference in eGFR in multivariable adjustment could simply arise from the imprecision introduced by the multivariate model, meaning that a true relationship might exist.

The pattern of consistency of the association of the lipid traits with the 3 kidney traits (percent difference in eGFR, risk for eGFR < 60 mL/min/1.73 m^2, and percent difference in ACR) is where the complexity becomes further apparent. In the case of HDL-C, there is, as one might expect, a directionally consistent relationship of HDL-C concentration with percent difference in eGFR, risk for low eGFR, and percent difference in ACR. The consistency across these traits (although 2 are essentially marking the same entity; ie, eGFR) for HDL-C lends weight to a potential protective role of HDL-C in CKD. The same is not the case for LDL-C or TG, for which both traits appear to associate with higher percent differences in both eGFR and ACR, potentially indicating a physiologic phenomenon for which there is higher filtration yet deteriorating function.

This study raises several questions about how to reliably interpret these various strands of evidence. Undoubtedly the main challenge in the wake of abundant genome-wide data and large-scale resources such as the UK Biobank is how to address the potential for genetic pleiotropy to confound the estimates derived from MR. At a June 2017 MR conference hosted by the Medical Research Council Integrative Epidemiology Unit in Bristol, United Kingdom, more than 30 MR methodologies were presented, the majority of which are new and have yet to be subjected to the same scrutiny as those that are becoming more established in the MR field.[24] Although exciting for those of us active in this field, it also poses major challenges; for example, which approaches should we use in our battery of tests when conducting MR, and what is the added value of the newer methodologies? This will no doubt be the subject of many narrative reviews to follow, but allow us to synthesize a few points below, based on those MR approaches that are now commonly used.

In the absence of genetic pleiotropy (the scenario in which ≥1 genetic variant used in a genetic instrument associates with >1 phenotype, described in detail in a recent review[19]), the conventional MR estimate ought to provide a reliable guide to the causal relationship of an exposure on an outcome. Established sensitivity analyses include MR-Egger–,[22] median-,[23] and mode-based[24] approaches. Each has its own assumptions on the type and amount of genetic confounding (see Table 5 in Hartwig et al[24]), meaning that if

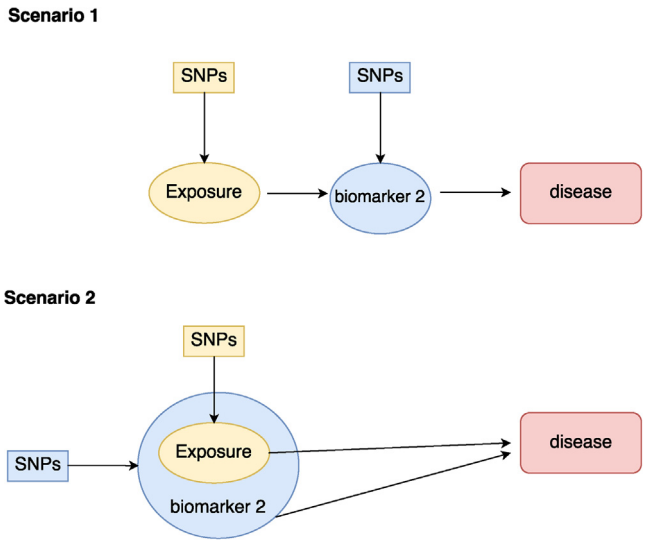

Figure 1. Challenges in interpreting multivariable Mendelian randomization (MR) analyses. Scenario 1: use of multivariable MR when the second biomarker (in addition to the exposure) lies on the causal pathway to disease. Adjusting for a potential mediator as a form of mediation analysis is suboptimal because error terms in the exposure to intermediate relationship are not properly accounted for in the analysis. Scenario 2: use of multivariable MR when the second biomarker measures the same entities as the primary exposure. Adjusting an exposure for an overlapping trait has the net effect of autoadjusting, meaning that the findings from multivariable MR are unreliable. In scenario 1, biomarker 2 is a mediating phenotype of the exposure (eg, investigating the degree to which triglycerides mediate the relationship of body mass index with risk for coronary heart disease[43]); in scenario 2, biomarker 2 is an overlapping trait with the exposure (eg, assessing the role of triglycerides and non–high-density lipoprotein cholesterol in risk for coronary heart disease[38]). Abbreviation: SNP, single-nucleotide polymorphism.