Mendelian randomization with multiple exposures: The importance of thinking about time

JA Labrecque[1]*, SA Swanson[1]

[1]Department of Epidemiology, Erasmus MC, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands.

E-mail: j.labrecque@erasmusmc.nl

*Corrseponding author: Jeremy A Labrecque

Have you ever wondered how Mendelian randomization (MR) studies can estimate a lifetime effect when the exposure is only measured once (1)? This is incredible considering that other familiar methods (2) would require that the exposure (and time-varying covariates) be measured repeatedly and frequently throughout the life-course to estimate the same effect. MR avoids this by making important assumptions about time to estimate effects. For example, the assumption that the relationship between the genetic variant(s) and the exposure is constant through time (3) allows the estimation of a lifetime effect (as defined in Table 1) even when exposure is only measured once. Regardless of the methods used to infer causality, it is not possible to define a causal effect or hypothesis without thinking about time. This may be even more true for MR than for conventional methods because of the potentially long periods of time between when the proposed instruments are set and the exposure and outcome are measured (4).

[Table 1 here]

MR studies increasingly use multiple instruments, multiple outcomes and, as can be seen in recent publications, multiple exposures (5,6). The incorporation of multiple exposures in MR adds another wrinkle, however: is it possible to capture longitudinal relationships between variables without longitudinal data? In short, what assumptions do we have to make about time in order to use MR to estimate causal effects which involve the relationship between multiple exposures? And what causal effects are we even estimating?

Here, we define lifetime effects for the parameters estimated with three of the more recently developed methods for incorporating multiple exposures into an MR framework: network MR (7), multivariable MR (8) and factorial MR (5). Network MR uses MR to estimate the effect of the exposure on the mediator but also the effect of the mediator on the outcome. Multiplying

these estimates results in an estimate of the indirect effect of the exposure on the outcome. Multivariable MR can estimate the direct effect of more than one variable on an outcome as long the MR assumptions hold for the collection of proposed instruments and exposures rather than having to hold for each variable individually. Factorial MR is similar to multivariable MR but can additionally estimate an interaction parameter between exposures. Adapting simulations from publications developing or using these methods, we demonstrate which longitudinal relationships can lead to biased estimates.

To ground these pertinent points in an empirical example, we will consider the recent study by Sanderson and colleagues (6), in which the investigators used multivariable MR to study the effects of education and cognitive ability on body mass index. A priori, it seems reasonable to suspect that pre-education cognitive ability has an effect on education and education has an effect on post-education cognitive ability. However, such bidirectional relationships between the exposures are not readily encoded in the theory grounding multivariable MR, as the theory (like many MR methods) is really only developed for time-fixed exposures. So, does incorporating time into this example change our interpretation of the results or the likely validity of the estimates?

Interpretation

Thinking about variables as time-fixed or point exposures in the context of MR is a simplification that can be valid in special circumstances but often obscures important relationships that should not be ignored. Considering lifetime effects in the context of network, multivariable and factorial MR, therefore, requires extending our thinking from one time point (Figure 1a) to multiple time points (Figure 1b). Even just increasing from one time point to two

greatly increases the number of possible relationships between the exposures themselves and the outcome. For example, it allows the relationship between A and B to be bidirectional and also allows each variable to have a direct effect on the outcome at more than one point in time.

[Figure 1 here]

One definition of a total lifetime effect of a variable is the effect of shifting the trajectory of the exposure by one unit on the outcome measured at a specific point in time (Table 1). The goal of network and multivariable MR is to decompose this total effect into a direct effect of A on Y (or multiple direct effects of different variables in multivariable MR) and, in the case of network MR, an indirect effect. The analogous direct effect of A on Y in Figure 1b, as estimated by both network and multivariable MR, is the effect of shifting the trajectory of A by one unit while holding B to a specific trajectory (and vice-versa for the direct effect of B) (5). The indirect effect is the effect of shifting the trajectory of the "mediator", B in Figure 1b, by the amount it would have shifted as a result of shifting A by one unit. It should be noted that both network and multivariable MR assume no interaction between variables A and B, that is, that the effect of each variable does not vary within strata of the other on the additive scale. The main effect for variable A in factorial MR is equivalent to the lifetime direct effect defined above but setting the trajectory for B to zero (and vice-versa for variable B). The interaction is then the effect of lifetime joint exposure minus the independent effect of variables A and B (i.e. while holding the other variable at baseline level).

Simulations

With well-specified definitions of the longitudinal parameters in mind, we can simulate data to determine whether these parameters are identifiable with MR-based approaches. We adapted

previously-published simulations to a setting with exposures varying over two time points (Figure 1): (i) Burgess et al (7) for network MR; (ii) the mediation scenario simulation published in Sanderson et al (6) and (iii) the Sanderson et al mediation scenario but with an interaction between A and B for factorial MR. The gray edges in Figure 1 were varied between 0 and 0.5 to whether they biased the estimates from each method. We chose not to include direct effects from the genetic variants to each variable at time 1 to avoid inducing bias due solely to time-varying relationships between the genetic variants and the exposures (3). For the same reason, we set the effect of each variable at time 0 on the same variable at time 1 to be equal to 1. G_A and G_B each represent one genetic variant for the network MR scenario and 10 variants each for the multivariable MR and factorial MR simulations. An additional ten variants were related to both A and B were added to the multivariable and factorial MR simulations. The network MR simulations had n=5000 (to match the original article) and all other simulations had n=30000 and all were run for 1000 iterations. A full description of the simulations and code can be found in the Supplementary methods and a full set of results can be found in the Supplementary results.

Table 2 shows the results for network MR. When none of the gray arrows are added, the simulation is equivalent to the time-fixed scenario and is unbiased. The total effect is biased when the B_0 to A_1 arrow is added. The direct and indirect effects are biased in all other scenarios except when only the arrow from A_0 to B_1 is added. In the biased scenarios, the indirect effect is always overestimated and the direct effect is always underestimated but the direction of the bias will depend on the direction of the effect of the simulation parameters generating the bias.

MR methods also be used to test causal hypotheses rather than to estimate the magnitude. Network MR also requires additional assumptions even when only the null hypothesis of no effect of both the indirect and direct effects is being tested (as opposed to estimating the

magnitude of effects). When *B* has no direct effect on *Y* (i.e. no indirect effect), network MR will falsely detect an indirect effect when *B* has an effect on *A* (when B_0 has a causal effect on A_1in our simulation). Therefore, testing the null hypothesis of no indirect effect, network MR requires the IV assumptions for each variable as well as an additional assumption that the mediator does not also cause the exposure. When *A* has no direct effect on *Y* (i.e. no direct effect), network MR will falsely detect a direct effect when there is an effect of the history of the mediator (when B_0has a direct effect on *Y* in our simulation). Therefore, testing the null hypothesis of no direct effect requires the additional assumption that the mediator, at the time measured, captures the entire mediated effect.

[Table 2 here]

Table 3 shows the results for the multivariable MR simulations when variable A causes variable B at each time point. Unlike network MR, multivariable MR remains unbiased when feedback between A and B is added as long as there is no direct effect of variables A and B at time 0. The effect of A is biased in all scenarios when direct effects of the exposures at time 0 are added. The effect of variable B is only biased when the arrow from A_0 to B_1 is added. The pattern of biases changes with different data generation mechanisms (*e.g.*, if B causes A or if there is no relationship between A and B). Multivariable MR sometimes requires extra assumptions to test null hypotheses that will depend on the relationship between variables *A* and *B*. If variable A does not cause variable B, no additional assumptions are required to test the null hypothesis of a direct effect of A on Y. If variable A causes B, then an assumption that variable B does not direct cause Y other than the time at which it was measured. [Table 3 here]

As mentioned previously, both network and multivariable MR are not valid when there is an interaction between A and B even in the time-fixed cases. To examine factorial MR in the longitudinal setting, we added interactions to the simulation used in the multivariable MR simulations (results in the Supplementary results). When an interaction between variables A and B was added only at time 1, factorial MR correctly estimated the interaction and the pattern of bias in the main effects was identical to that observed in multivariable MR. When an additional interaction was added to the baseline effects (when present), factorial MR was biased in all scenarios except when A and B do not causes each other at any time.

Conclusion

Understanding time is crucial to understanding MR, which becomes increasingly clear when the MR analysis involves multiple exposures of interest that may have complex relationships with one another over time. The MR methods examined here—network, multivariable and factorial MR—work under some but not all longitudinal causal structures. Intuitively, this happens because feedback (or interaction) between variables A and B makes it more difficult for these models to tease apart their independent effects. Returning to the Sanderson et al [Table 3 in reference (6)] example of cognitive ability, we can simulate data with a similar causal structure to see whether we would expect bias when cognitive ability both causes and is subsequently caused by education (Supplementary results). We find that the effect of cognitive ability is unbiased but that the effect of education on BMI requires there to be no direct effect of cognitive ability on BMI before the age of educational attainment. Therefore, if this were the true causal structure, we would expect that the degree of bias of the effect of education is tied to the degree to which cognitive ability before the age of educational attainment directly effects BMI. The

degree of bias more generally due to ignoring time in MR can be further evaluated using previous simulations (3).

Because the bidirectional relationship between variables A and B is responsible for many of the biases described here, bidirectional MR may be of use to detect whether the relationship between A and B is bidirectional in order to inform further analyses. We would caution researchers, however, that when the relationsip between A and B is truly bidirectional, two tests with 80% power will correctly identify the bidirectionality only $80\% * 80\% = 64\%$ of the time. Therefore, only high-powered bidirectional MR studies will be able to confidently eliminate the possibility of bidirectional effects. Moreover, traditional power analyses in MR are not yet parameterized for time-varying exposures (9).

When there is feedback between variables, it should come as no surprise that disentangling their effects on an outcome will almost always require measuring them repeatedly, regardless of the analytic method used. We suggest that researchers wishing to use these MR methods for multiple exposures think carefully about their causal question and whether the relationship between the two variables of interest is likely to invalidate (or at least complicate) the MR method chosen.

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Figure 1— Causal graph with (a) time-fixed exposures (left) and (b) time-varying exposures (right). G indicates genetic variants with subscripts indicating which variables they are associated with. A and B are the exposures of interest (B is considered the mediator for the network MR example), Y is the outcome and U is an unmeasured confounder. Numerical subscripts are used to show when a variable is measured in time.

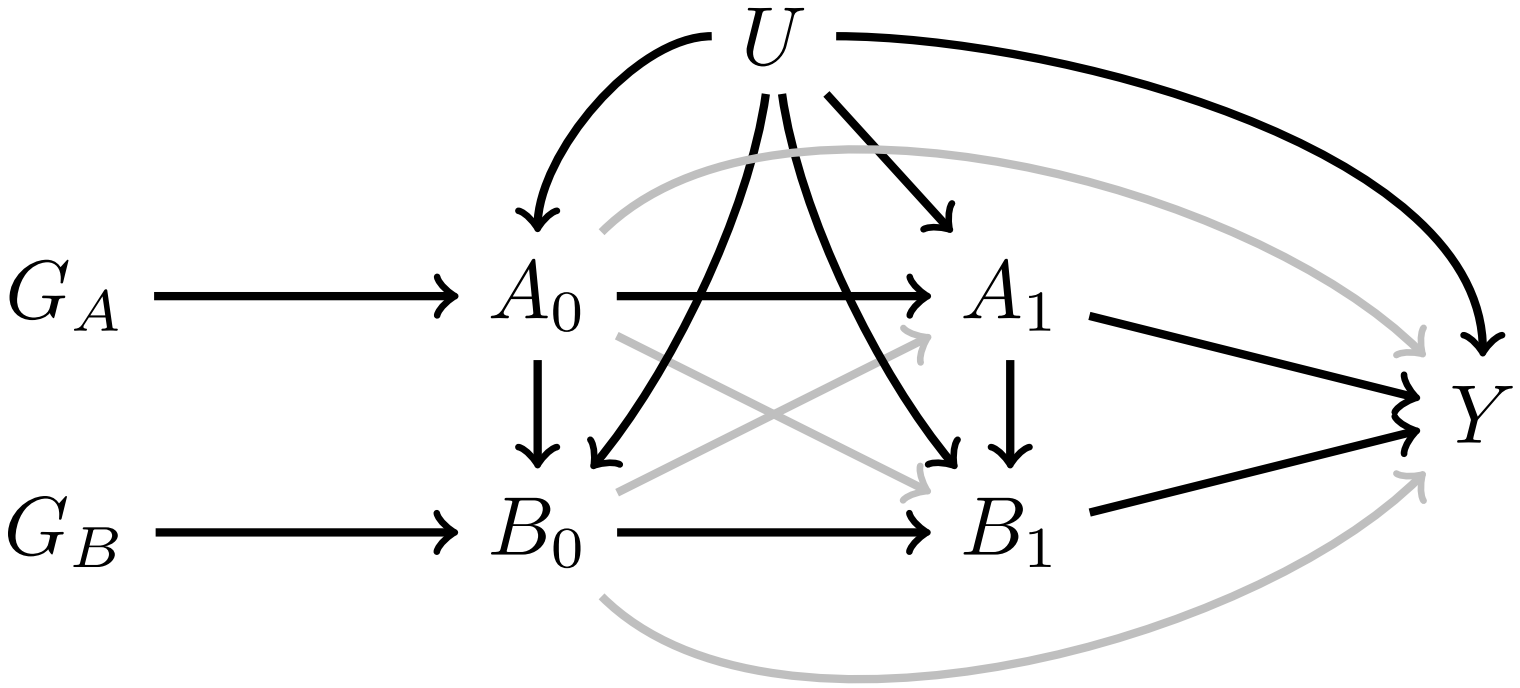
U
G_A
A_0
A_1
Y
G_B
B_0
B_1

Table 1—A description of the estimands of various MR analyses as well as counterfactual definitions.

Type	Estimand	Counterfactual definition
Classic MR	Total effect	$E[Y_k^{\bar{a}+\bar{1}}] - E[Y_k^{\bar{a}}]$
Network MR	Direct effect	$E[Y_k^{\bar{a}+\bar{1},\bar{b}}] - E[Y_k^{\bar{a},\bar{b}}]$
	Indirect effect	$E[Y_k^{\bar{a},\bar{B}^{\bar{a}+\bar{1}}}] - E[Y_k^{\bar{a},\bar{B}^{\bar{a}}}]$
Multivariable MR	Direct effect of A	$E[Y_k^{\bar{a}+\bar{1},\bar{b}}] - E[Y_k^{\bar{a},\bar{b}}]$
	Direct effect of B	$E[Y_k^{\bar{a},\bar{b}+\bar{1}}] - E[Y_k^{\bar{a},\bar{b}}]$
Factorial MR	Main effect of A	$E[Y_k^{\bar{a}+\bar{1},\bar{b}=0}] - E[Y_k^{\bar{a},\bar{b}=0}]$
	Main effect of B	$E[Y_k^{\bar{a}=0,\bar{b}+\bar{1}}] - E[Y_k^{\bar{a}=0,\bar{b}}]$
	Interaction of A and B	$E[Y_k^{\bar{a}+\bar{1},\bar{b}+\bar{1}}] - E[Y_k^{\bar{a}+\bar{1},\bar{b}}] - E[Y_k^{\bar{a},\bar{b}+\bar{1}}] + E[Y_k^{\bar{a},\bar{b}}]$

Legend: Y-outcome, a-variable a, b-variable b, k-time at which the outcome is measured, variables with an overbar indicate a vector of values from conception to time k

Table 2—Estimates and bias using network MR to estimate longitudinal parameters from simulated data with exposure and mediator measured at two time points.

				Total effect			Direct effect			Indirect effect		
$A_0 \longrightarrow Y$	$B_0 \longrightarrow Y$	$A_0 \longrightarrow B_1$	$B_0 \longrightarrow A_1$	True	Estimated	Bias	True	Estimated	Bias	True	Estimated	Bias
0.0	0.0	0.0	0.0	3.0	3.0	0.0	1.0	1.0	0.0	2.0	2.0	0.0
0.0	0.0	0.0	0.5	3.0	2.7	-0.3	1.0	0.4	-0.6	2.0	2.2	0.2
0.0	0.0	0.5	0.0	3.5	3.5	0.0	1.0	1.0	0.0	2.5	2.5	0.0
0.0	0.0	0.5	0.5	3.5	3.0	-0.5	1.0	0.3	-0.7	2.5	2.7	0.2
0.5	0.5	0.0	0.0	4.0	4.0	0.0	1.5	1.0	-0.5	2.5	3.0	0.5
0.5	0.5	0.0	0.5	4.0	3.3	-0.7	1.5	0.6	-0.9	2.5	2.8	0.3
0.5	0.5	0.5	0.0	4.5	4.5	0.0	1.5	0.8	-0.7	3.0	3.7	0.7
0.5	0.5	0.5	0.5	4.5	3.7	-0.8	1.5	0.3	-1.2	3.0	3.3	0.3

Table 3—Estimates and bias using multivariate MR to estimate longitudinal parameters from simulated data with exposure and mediator measured at two time points in a scenario where variable B mediates the effect of variable A (Mediation).

				Variable A			Variable B		
$A_0 \longrightarrow Y$	$B_0 \longrightarrow Y$	$A_0 \longrightarrow B_1$	$B_0 \longrightarrow A_1$	True	Estimated	Bias	True	Estimated	Bias
0.0	0.0	0.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0
0.0	0.0	0.0	0.5	1.0	1.0	0.0	1.0	1.0	0.0
0.0	0.0	0.5	0.0	1.0	1.0	0.0	1.0	1.0	0.0
0.0	0.0	0.5	0.5	1.0	1.0	0.0	1.0	1.0	0.0
0.5	0.5	0.0	0.0	1.5	1.0	-0.5	1.5	1.5	0.0
0.5	0.5	0.0	0.5	1.5	0.8	-0.8	1.5	1.5	0.0
0.5	0.5	0.5	0.0	1.5	1.3	-0.3	1.5	1.3	-0.3
0.5	0.5	0.5	0.5	1.5	1.0	-0.5	1.5	1.3	-0.2