Challenges to interpretation of Mendelian randomization estimates with time-varying exposures

Appendix A: General proof of MR with time-varying exposures using structural models

We have previously defined the lifetime effect of increasing a time-varying A by one unit as:

$$E[Y_K^{\bar{A}+\bar{1}} - Y_K^{\bar{A}}]$$

where Y is the outcome, $\bar{A}$ is exposure history and K is the age when the outcome and exposure are measured. Here, we will demonstrate under which conditions MR estimates can be interpreted as this type of lifetime effect. Time-fixed and time-varying exposures will be addressed separately.

Time-fixed A

When A is time-fixed, our counterfactual definition of a lifetime effect of increasing A by one unit is:

$$E[Y_K^{A+1} - Y_K^{A}]$$

Our structural causal model for $E[Y_K^A]$ is:

$$E[Y_K^A] = \gamma_0 + \int_0^K \gamma_A(t) A \mathrm{d}t$$

where $\gamma_0 = E[Y_K^{a=0}]$ and $\gamma_A(t)$ is the potentially time-varying effect of A on Y_K^A. We can use this structural model to define the lifetime effect:

$$\begin{aligned} E[Y_K^{A+1} - Y_K^A] &= \gamma_0 + \int_0^K \gamma_A(t)(A+1)\mathrm{d}t - \gamma_0 - \int_0^K \gamma_A(t) * A \mathrm{d}t && (1) \\ &= \int_0^K \gamma_A(t) * (A+1)\mathrm{d}t - \int_0^K \gamma_A(t) * A \mathrm{d}t && (2) \\ &= \int_0^K \gamma_A(t)\mathrm{d}t && (3) \end{aligned}$$

When A is time-fixed, we can use the following structural equation:

$$E[A^G] = \beta_0 + \beta_G * G$$

where $\beta_0 = E[A^{g=0}]$, G is a binary genetic instrument and β_G is the effect of the G on A.

We can use the structural model for $E[A^G]$ in the structural model for $E[Y_K^A]$ to obtain the reduced form estimate:

$$E[Y_K^{g=1}] - E[Y_K^{g=0}] = \gamma_0 + \int_0^K \gamma_A(t)(\beta_0 + \beta_G)\mathrm{d}t - \gamma_0 - \int_0^K \gamma_A(t)(\beta_0)\mathrm{d}t \tag{4}$$

$$= \int_0^K \gamma_A(t)\beta_G\mathrm{d}t \tag{5}$$

$$= \beta_G \int_0^K \gamma_A(t)\mathrm{d}t \tag{6}$$

We now divide the reduced form estimate by the effect of G on A, β_G:

$$= \frac{\beta_G \int_0^K \gamma_A(t)\mathrm{d}t}{\beta_G} \tag{7}$$

$$= \int_0^K \gamma_A(t)\mathrm{d}t \tag{8}$$

The results matches the lifetime effect from our earlier structural model. Therefore, when A is time-fixed, MR can be interpreted as a lifetime effect.

Time-varying A

We repeat the same procedure but with time-varying A. Our structural model for $Y_K^{\bar{A}}$ is identical to the structural model for Y_K^A except we now allow A to vary over time:

$$E[Y_K^{\bar{A}}] = \gamma_0 + \int_0^K \gamma_A(t)A(t)\mathrm{d}t$$

where $\gamma_0 = E[Y_K^{\bar{A}=\bar{0}}]$, $\gamma_A(t)$ is the coefficient of the exposure window at time t and $A(t)$ is the value of A at time t. With this structural model our lifetime effect of increasing $\bar{A}$ by 1:

$$E[Y_K^{\bar{A}+\bar{1}} - Y_K^{\bar{A}}] = \gamma_0 + \int_0^K \gamma_A(t)(A(t)+1)\mathrm{d}t - \gamma_0 - \int_0^K \gamma_A(t) * A(t)\mathrm{d}t \tag{9}$$

$$= \int_0^K \gamma_A(t)(A(t)+1)\mathrm{d}t - \int_0^K \gamma_A(t)(A(t))\mathrm{d}t \tag{10}$$

$$= \int_0^K \gamma_A(t) \tag{11}$$

Our structural model for A^G when A is time varying is:

$$E[A_T^G] = \beta_0 + \beta_G * G + \beta_T * T + \beta_{GT} * G * T$$

where $\beta_0 = E[A^{g=0}|T=0]$, β_G is the effect of G at $t=0$, β_T is the effect of t on those with $G=0$ and $\beta_T + \beta_{GT}$ is the effect of t on those with $G=1$. Using this structural model in the structural model for $Y_K^{\bar{A}}$ we can obtain the structural model for the reduced form estimate:

$$E[Y_K^{g=1} - Y_K^{g=0}] = \int_0^K \gamma_A(t)(\beta_0 + \beta_G + \beta_T * t + \beta_{GT} * t)\mathrm{d}t - \int_0^K \gamma_A(t)(\beta_0 + \beta_T * t)\mathrm{d}t \tag{12}$$

$$= \int_0^K \gamma_A(t)(\beta_G + \beta_{GT} * t)\mathrm{d}t \tag{13}$$

In a typical MR study, A is measured at the same time as Y (T=K).Therefore, When A is time-varying, the instrument strength will be measured at time K and will be:

$$E[A_K^{g=1}|T = K] - E[A_K^{g=0}|T = K] = \beta_0 + \beta_G + \beta_T * K + \beta_{GT} * K - (\beta_0 + \beta_T * K) \tag{14}$$

$$= \beta_G + \beta_{GT} * K \tag{15}$$

Dividing the reduced form by the genetic effect measured at time K we obtain:

$$\frac{E[Y_K^{g=1} - Y_K^{g=0}]}{E[A_K^{g=1} - A_K^{g=0}]} = \frac{\int_0^K \gamma_A(t)(\beta_G + \beta_{GT} * t)\mathrm{d}t}{\beta_G + \beta_{GT} * K} \tag{16}$$

Therefore, in this case the MR estimate has no recognizable interpretation and cannot be interpreted as the lifetime effect. For no value of K will MR return an unbiased effect of the lifetime estimate.

When A is time-varying but the genetic effect is constant with time (i.e. $\beta_{GT} = 0$), then the equation above reduces to the lifetime effect:

$$\frac{E[Y_K^{g=1} - Y_K^{g=0}]}{E[A_K^{g=1} - A_K^{g=0}]} = \frac{\int_0^K \gamma_A(t)\beta_G\mathrm{d}t}{\beta_G} \tag{17}$$

$$= \int_0^K \gamma_A(t) \tag{18}$$

When $\beta_{GT} \neq 0$ but the effect of A is cumulative, (i.e. γ_A is not time-varying) it is possible to obtain the lifetime effect another way. When the effect of A is cumulative, the integral in the reduced form equation becomes:

$$E[Y_K^{g=1} - Y_K^{g=0}] = \gamma_A(t) \int_0^K \gamma_A(t)(\beta_G + \beta_{GT} * t)\mathrm{d}t \tag{19}$$

$$= \gamma_A * K * [avg(A^{g=1}) - avg(A^{g=0})] \tag{20}$$

Therefore, if A is measured over many time points and it is possible to obtain an estimate of the average genetic effect, dividing by this value will return the average lifetime effect. In practice this would be quite difficult.

Appendix B: Code for simulation

```
require(pracma) ; require(magrittr) ; require(dplyr)

MR_longitudinal_sim2 <- function(GA_shape, exp_window_shape) {

  # Create age vector
  age <- seq(0, 50, length.out = 1001)

  # Set lifetime effect at age 50
  lifetime_effect_at_50=2

  # Function to create vector of G-A relation for one of four prespecified relationships
  GA_fn <- function(age, GA_shape) {
    if (GA_shape=="unif") {
      # Difference between homozygous alleles always 0.5
      return(rep(0.5, times = length(age)))
    } else if (GA_shape=="FTO") {
      # Difference between homozygous alleles follows a pattern roughly similar
      # to FTO (i.e. rough bell-shaped curve centred at age 25)
      return(dnorm(x = age, mean = 25, sd = 10)*15 + 0.2538)
    } else if (GA_shape=="incr") {
      # Difference between homozygous alleles increases with age
      return(0.01+0.9*age/50)
    } else if (GA_shape=="decr") {
      # Difference between homozygous alleles decreases with age
      return(1-0.9*age/50)
    } else {
      stop("Shape argument for GA_fn must be either unif, FTO, incr or decr")
    }
  }

  # Define the the exposure window
  exp_window_fn <- function(age, exp_window_shape) {

    if (exp_window_shape=="unif") {
      # The exposure window is constant over time. This is a cumulative model
      # where exposure has the same effect at any time
      return(rep(1, times = length(age)))
    } else if (exp_window_shape=="recent") {
      # Recent exposure has more effect (Note that the curve at 30 is shifted
      # later in the code.)
      return(dnorm(x = age, mean = 50, sd = 10))
    } else if (exp_window_shape=="critical") {
      # Importance of exposure increases with time
      return(dnorm(x = age, mean = 25, sd = 2))
    } else if (exp_window_shape=="incr") {
      # Importance of exposure decreases with time
      return(age/50)
    } else {
      stop("Shape argument for exp_window_fn must be either unif, recent, critical or incr")
    }
```

```
}

# Create BMI difference by genetic variant
BMI_diff <- GA_fn(age = age, GA_shape = GA_shape)

# Create exposure window
exp_window_wts_unscaled <- exp_window_fn(age = age, exp_window_shape = exp_window_shape)

# Scale exposure window to equal lifetime effect at 50
exp_window_wts <- lifetime_effect_at_50*exp_window_wts_unscaled/
  trapz(age, exp_window_wts_unscaled)


# Calculate lifetime effect of changing BMI trajectory by 1 unit at age 30 and
# age 50. (Note that the if statement shifts the exposure window to where it
# should be at age 30.)
est_lifetime_effect30 <- trapz(age[age<=30],
                               (BMI_diff[age<=30]+1)*exp_window_wts[age<=30]) -
  trapz(age[age<=30],
        (BMI_diff[age<=30])*exp_window_wts[age<=30])
if (exp_window_shape=="recent") {
  est_lifetime_effect30 <- trapz(age[age<=30],
                                 (BMI_diff[age<=30]+1)*exp_window_wts[age>=20]) -
    trapz(age[age<=30], (BMI_diff[age<=30])*exp_window_wts[age>=20])
}
est_lifetime_effect50 <- trapz(age, (BMI_diff+1)*exp_window_wts) -
  trapz(age, (BMI_diff)*exp_window_wts)

# Calculate the reduced form estimate at age 30 and age 50 (Note that the line
# with the if statement shift the exposures to where it should be for age 30.)
reduced_form_est30 <- trapz(age[age<=30], BMI_diff[age<=30]*exp_window_wts[age<=30])
if (exp_window_shape=="recent") {
  reduced_form_est30 <- trapz(age[age<=30],
                              BMI_diff[age<=30]*exp_window_wts[age>=20])
}
reduced_form_est50 <- trapz(age, BMI_diff*exp_window_wts)

# MR estimates at age 30 and age 50
MR_est30 <- round(reduced_form_est30/BMI_diff[age==30], 2)
MR_est50 <- round(reduced_form_est50/BMI_diff[age==50], 2)

# Absoulate bias at age 30 and age 50
abs_bias30 <- MR_est30 - est_lifetime_effect30
abs_bias50 <- MR_est50 - est_lifetime_effect50

# Relative bias at age 30 and age 50
rel_bias30 <- ((MR_est30/est_lifetime_effect30)-1)*100
rel_bias50 <- ((MR_est50/est_lifetime_effect50)-1)*100

# Arrange results
res <- round(c(est_lifetime_effect30, MR_est30, abs_bias30, rel_bias30,
               est_lifetime_effect50, MR_est50, abs_bias50, rel_bias50),2)
names(res) <- c(paste0(c("true", "MR", "abs", "rel"), 30),
```

```
                  paste0(c("true", "MR", "abs", "rel"), 50))

  return(res)

}

# Create data.frame of all combinations of G-A relationships and exposre windows
grid <- expand.grid(GA_shape=c("unif","FTO","incr","decr"),
                    exp_window_shape=c("unif","recent","critical","incr"))

ds <- do.call(rbind, mapply(grid[ ,1], grid[ ,2],
                            FUN=function(x,y) MR_longitudinal_sim2(GA_shape = x,
                                                                   exp_window_shape = y),
                            SIMPLIFY = FALSE)) %>% as.data.frame
ds$GA <- as.character(grid$GA_shape)
ds$exp_win <- as.character(grid$exp_window_shape)


ds %<>% dplyr::select(., GA, exp_win, dplyr::everything())
ds$GA <- rep(c("Time-fixed", "FTO", "Increasing", "Decreasing"),4)
ds$exp_win <- rep(c("Uniform", "Recent", "Critical", "Increasing"), each=4)
ds <- ds[order(match(ds$GA,c("Time-fixed", "FTO", "Increasing", "Decreasing"))),]
row.names(ds) <- NULL
names(ds)[1:2] <- c("GA shape", "Exposure window")
names(ds)[names(ds) %in% c("rel30","rel50")] <- c("rel30 (%)", "rel50 (%)")
```

Appendix C: Reverse causality and bi-directional MR studies

One often cited advantage of MR over traditional observational research is its invulnerability to reverse causation (1). This characteristic of MR is used to investigate bidirectional relationships between two variables (2,3). Note that concern about the direction of causality between two variables requires that both be time-varying. A time-fixed variable cannot be caused by another variable.

Figure 1 illustrates a causal graph with a bi-directional relationship between A and Y. G_A and G_Y represent genetic instruments for A and Y respectively. A significance test of the sharp null of no effect of A on Y at any time can be carried out by testing whether Y differs by genetic variant G_A.

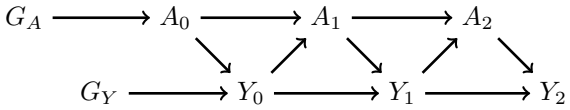

Figure 1: Causal graph demonstrating MR studies looking at bi-directional relationships.

If the relationship is unidirectional, the two null-hypothesis tests (one in each direction) will correctly identify the direction of the relationship. The MR estimate will also estimate the lifetime effect if the instrument strength is constant. If the relationship is bidirectional, both null-hypotheses will be rejected correctly identifying the bidirectional relationship (4). In the bidirectional case, however, the instrument strength for both A and Y are almost guaranteed to be time-varying. This is because for G_A to have the same effect on A_0 as it does on A_1. the direct path from A_0 to A_1 must balance the path from A_0 to Y_0 to A_1. This type of balancing of effect is unlikely. Therefore, when bi-directional MR suggests a bi-directional relationship, both estimates will be biased and, therefore, cannot be used for effect estimation or to determine the dominant direction of the relationship.

Appendix D Using MR to estimate the effect of point exposures

We have established that if the effect of the genetic variant varies with time, MR estimates cannot be interpreted as lifetime effects. We now investigate the conditions under which the MR estimate could be interpreted as the effect of a point exposure.

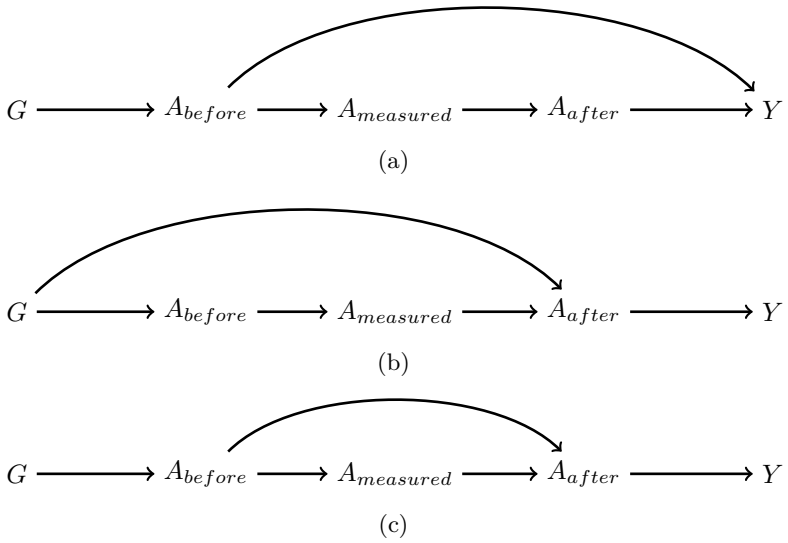

Figure 2: Three causal graphs showing how the exclusion restriction can be violated when estimating a point intervention of a time-varying exposure. This exclusion restriction violation with bias the MR estimate even when the exclusions restriction holds for A as a whole The causal graphs show the relationship between a genetic variant (G), a time-varying exposure when it was measured as well as before and after measurement, and an outcome (Y).

We can adapt the standard IV causal graph (Figure **??**) for time-varying A by splitting it into different nodes representing A at different points in time (Figure 2). Here, A is divided into three nodes to represent the value of the A when it was measured ($A_{measured}$), before it was measured (A_{before}) and after it was measured (A_{after}). This is a crude way of depicting a time-varying variable on a causal graph because A_{before} and A_{after} could be divided into many separate nodes but is sufficient for the purposes of our demonstration. We suppress U from further causal diagrams but it can always be assumed to exist and affect all nodes except G.

When using MR to estimate the effect of point intervention, it is possible for the exclusion restriction to hold for A as a whole but not for A at a specific time point. There are three plausible mechanisms that can violate the exclusion restriction for the effect $A_{measured}$ on Y. The first is a direct effect of A_{before} on Y (Figure 2a). Whether or not this is true will depend on when the exposure is measured relative to the relevant exposure window. For example, for exposures with only short term effects such as the effect of BMI on bone density, it would be possible to measure BMI before the relevant exposure window which would mean that BMI previous to when it was measured would have no direct effecton bone density. For exposures with cumulative, long term effects, such as the effect of BMI on cancer, measuring BMI before the relevant exposure window may be more difficult.

The second mechanism is the longitudinal effect of a genetic variant. Genetic variants do not only affect phenotypes at conception, as we have seen, but can have effects that vary over time. If the genetic variant affects the exposure after it has been measured, this will also violate the exclusion restriction (Figure 2b). This may occur, for example, with genetic variants whose direction of effect on blood pressure changes with time (5). If blood pressure is measured in early adulthood and the outcome is measured in the elderly, the effect of the genetic variant on blood pressure after it was measured would be a violation of the ER.

The last mechanism occurs when A_{before} has an effect on A_{after} that does not pass through the measured

exposure (Figure **??**). This would occur if the exposure is in a feedback loop with another variable as was seen in Appendix C.

Note that in all these cases, the exclusion restriction holds for A as a whole. That is, the entire effect of the G on Y passes through A. When we consider A measured at a specific time, however, the exclusion restriction does not hold.

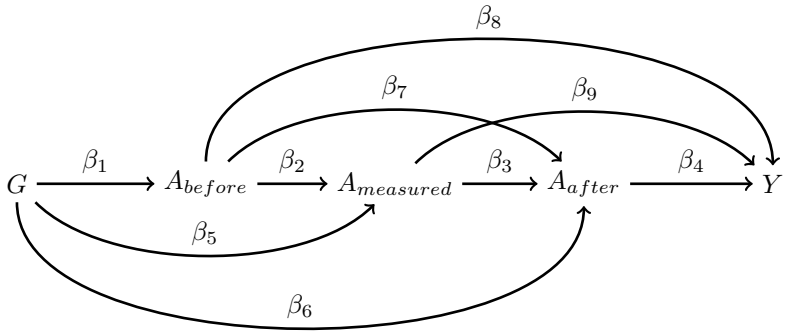

Figure 3: DAGs with time-varying exposures that violate the exclusion restriction

We can further calculate the magnitude of the bias with time-varying exposures and formalize the additional assumptions required for unbiased MR estimates with time-varying exposures. Figure 3 is a causal diagram of the relationship between a a gene, G, a time-varying exposure, E, and an outcome Y. As before, the exposure node has been divided to represent exposure at different points in time. Here we have divided A into $A_{measured}$ which represents the value of the exposure when it was measured, A_{before} which represents the value of the exposure before it was measured and A_{after} which represents the value of the exposure after it was measured. The causal diagram includes all possible relationships between these variables except a direct effect of G on Y because we are assuming that the exclusion restriction holds for A as a whole.

Each edge is labeled with a β representing the effect of one variable on another. We can use these βs to calculate the effect of G on Y, the effect of G on $A_{measured}$ and the ratio of these quantities which is the MR estimate of the effect of $A_{measured}$ on Y:

$$MR_{A_{measured},Y} = \frac{\beta_1[\beta_2(\beta_3\beta_4 + \beta_9) + \beta_4\beta_7 + \beta_8] + \beta_4\beta_6 + \beta_5(\beta_3\beta_4 + \beta_9)}{\beta_1\beta_2 + \beta_5}$$

By collecting and canceling terms, this equation simplifies to:

$$MR_{A_{measured},Y} = \underbrace{\beta_3\beta_4 + \beta_9}_{\text{True value}} + \underbrace{\frac{\beta_1\beta_8 + \beta_4(\beta_1\beta_7 + \beta_6)}{\beta_1\beta_2 + \beta_5}}_{\text{Bias}}$$

The first term is the true value of the MR estimate and the second is the bias term. The MR estimate will be unbiased when the bias term is equal to zero. Ignoring the possibility of terms canceling out, there are four ways to make bias term to be equal to zero all requiring at least two βs to be equal to zero. The first is that β_6, β_7 and β_8 are all zero. In other words, A_{after} is not directly affected by G, A_{before} does not directly affect the outcome and A_{before} only affects A_{after} through its effect on $A_{measured}$. The second is that β_1 and β_6 are zero meaning that G only affects $A_{measured}$ and not before or after. The third way assumes β_4 and β_8 are zero meaning that the exposure only affects the outcome at time t and not before or after. The last way assumes that edges 1 and 8 are zero meaning that G does not affect the A before time t and the exposure does not affect the outcome after time t.

The first situation requires a linear chain of causation between the gene, exposure and outcome which may be plausible when the exposure is the gene product itself and the gene product has very short-lived affects.

The latter three situations where the bias is zero all rely on measuring the exposure at precisely the right moment to avoid any bias. Measuring the exposure at such a precise moment would require a lot of a priori knowledge about longitudinal effects of the gene and exposure. We find these scenarios unlikely.

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