Mendelian Randomization and MR-DoC2

HGEN619

Luis Castro-de-Araujo[a]

11/28/2022

Virginia Institute for Psychiatric and Behavioral Genetics

[a] Post-doc T32. luis.araujo@vcuhealth.org

Introduction to mendelian randomization

Sensitivity analysis in MR

Mixing with the DoC model

Bi-directional MR-DoC, MR-DoC2

Introduction to Mendelian randomization

Intro

- Problems with observational data
- Randomized controlled trials
- Mendelian Randomization (MR):
  - How it works
  - Core assumptions
  - Calculating causal effect estimates
- MR example
- Limitations of MR

Thanks David Evans

Lot's of slides borrowed from David Evans' talk[a]

[a] Evans, June 2021, "Introduction to Mendelian Randomization - Part 1," (Boulder 2021).

The Problem with Inferring Causality in Observational Studies

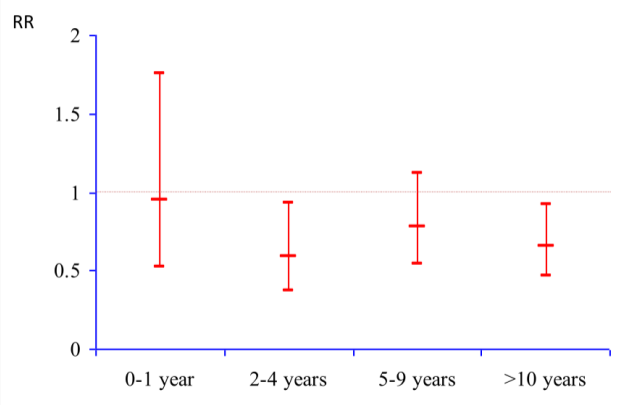
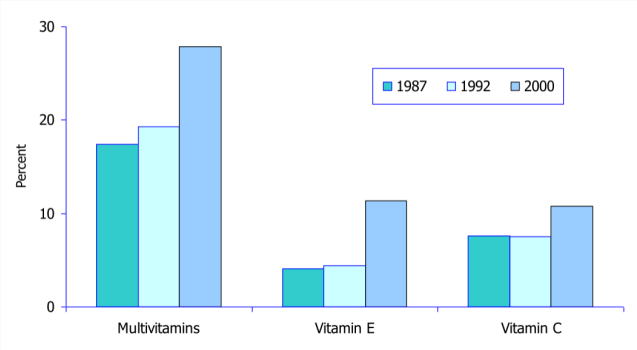


Figure 1: CHD risk according to duration of current Vitamin E supplement use compared to no use

[1] Rimm, (1993), "Vitamin E Consumption and the Risk of Coronary Heart Disease in Men," *N Engl J Med*.

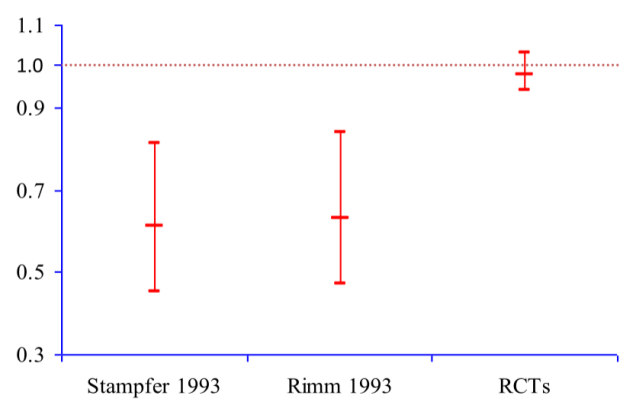
USE OF VITAMIN SUPPLEMENTS BY US ADULTS, 1987-2000



2

[2] Millen, (2004), "Use of Vitamin, Mineral, Nonvitamin, and Nonmineral Supplements in the United States," *J Am Diet Assoc.*

Vitamin E supplement use and risk of Coronary Heart Disease



3

[3] Stampfer, (1993), "Vitamin E Consumption and the Risk of Coronary Disease in Women," *N Engl J Med*; Eric B. Rimm, Meir J. Stampfer, Alberto Ascherio, Edward Giovannucci, Graham A. Colditz, and Walter C. Willett, "Vitamin E Consumption and the Risk of Coronary Heart Disease in Men"; Eidelman, (2004), "Randomized Trials of Vitamin E in the Treatment and Prevention of Cardiovascular Disease," *Arch Intern Med*.

MANY OTHER EXAMPLES

- VITAMIN C, VITAMIN A, HRT,
- MANY DRUG TARGETS.....
- WHAT'S THE EXPLANATION?

Vitamin E levels and confounding risk factors:

Reduces Vitamin E levels

- Childhood SES[a]
- Manual social class
- No car access
- State pension only
- Smoker
- Obese

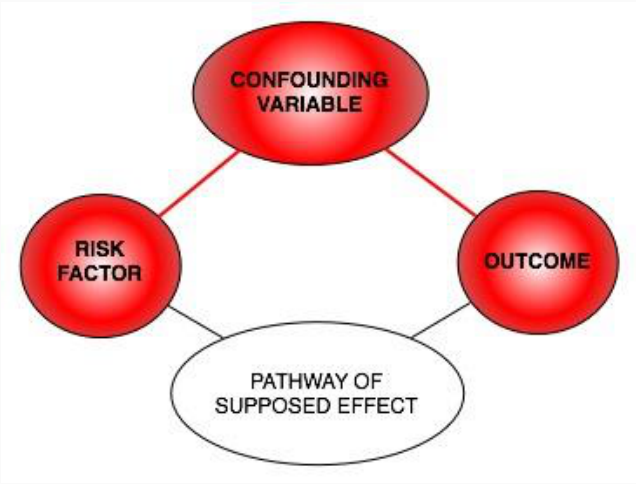
Increases Vitamin E levels

- Daily alcohol
- Exercise
- Low fat diet
- Height
- Leg length

[a] Lawlor, (2004), "Those Confounded Vitamins," *Lancet*.

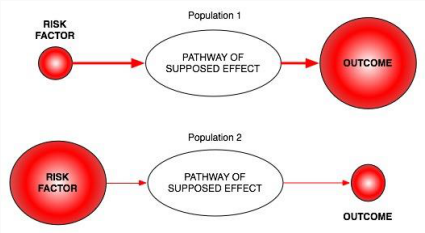
CLASSIC LIMITATIONS OF OBSERVATIONAL STUDIES

- Confounding

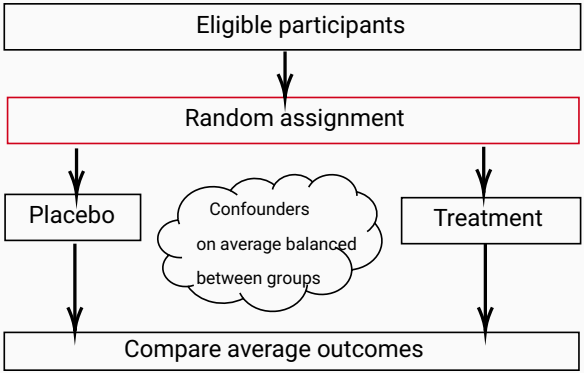


- Reverse causation

- Bias



Causal inference, gold standard: randomized controlled trial[5]



[4] Camelia, 2017, "Mendelian Randomization," (VIPBG 2017).

[5] Camelia, 2017, "Mendelian Randomization," (VIPBG 2017).

The Need for Observational Studies

Randomized Controlled Trials (RCTs):

- Not always ethical or practically feasible eg anything toxic
- Expensive, requires experimentation in humans
- Impractical for long follow up times
- Should only be conducted on interventions that show very strong observational evidence in humans

Observational studies:

- Association between environmental exposures and disease measured in observational designs (non-experimental) eg case-control studies or cohort studies
- Reliably assigning causality in these types of studies is ***very limited***

Hierarchy of observational and experimental data

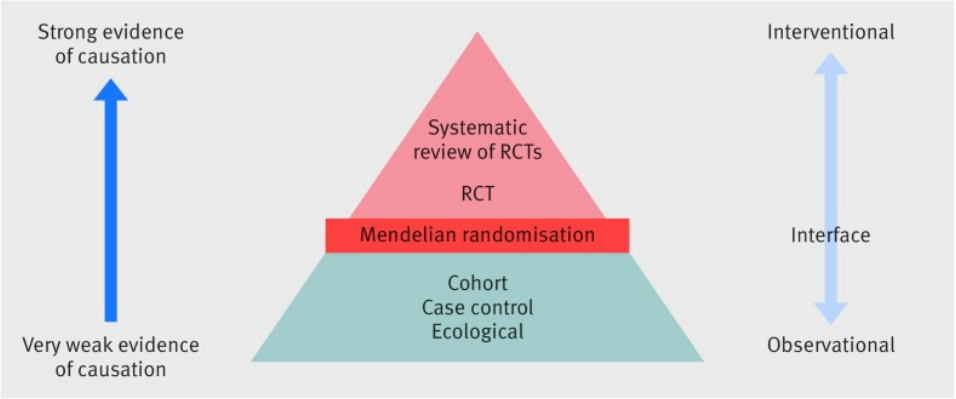


Figure 2: Mendelian randomisation studies sit at the interface of experimental and observational studies. Their findings can be used to provide more reliable evidence to guide interventional research and provide information about potential public health interventions when a randomised controlled trial may not be feasible.

[6] Davies, (2018), "Reading Mendelian Randomisation Studies," *BMJ*.

Philip G Wright

- Known for first description of the parameter identification problem
- The instrumental variables method[a]
- Could supply and demand be derived from price/volume data? As price confounds both supply & demand, Wright deduced that to resolve them, he needed an “instrument” – Something affecting only one of demand or supply. With this, he could compute the curves he needed.
- Wright hit upon variation in rainfall. Rain didn’t alter demand, but by increasing grass production, did impact butter supply. And thus the need to model food supply saw the first instrumental model conceived in 1938!
- Sewell Wright’s dad!

[a] Timothy Bates [@timothycbates], October 2022, “Using a Modern SEM-based IV Model & Wanted to Learn Who Invented Them. Turns Out Likely Sewell Wright’s (SEM) Dad! In a Situation Similar to "Student" Wright Had a Commercial Need,” *Twitter*, (2022).

The Wide Applicability of MR

- Traditional Observational Epidemiological Studies
- Behavior Genetics and the Social Sciences
- Molecular Studies
- Pharmacogenomics

How does Mendelian randomization work?

What does MR do?

- Assess causal relationship between two variables
- Estimate magnitude of causal effect

How does it do this?

- By harnessing Mendel's laws of inheritance

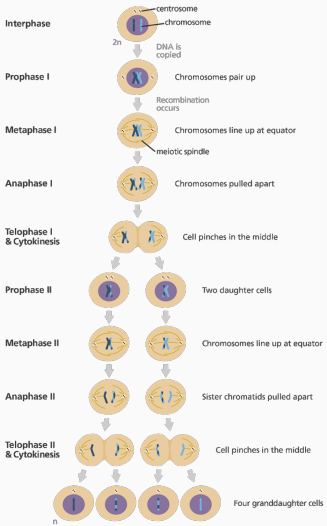
Gregor Mendel

Mendel's Laws of Inheritance

1. Segregation: alleles separate at meiosis and a randomly selected allele is transmitted to offspring
2. Independent assortment: alleles for separate traits are transmitted independently of one another

- Mendel in 1862

Meiosis



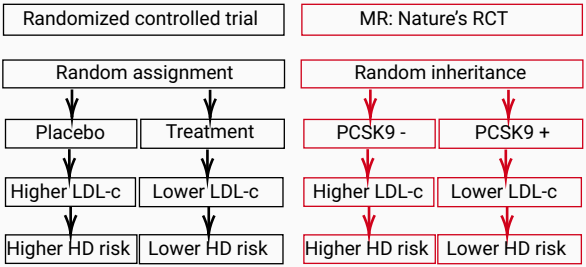
- Two cell divisions
- Produces four daughter cells
- Produces haploid cells
- Daughter cells are non-identical
- Produces sex cells
- Crossing-over occurs
- Homologous chromosomes pair up
  - and are separated at random (independent segregation)

Genetic variant as an instrument

The genotype only affects the disease status indirectly and is assigned randomly (given the parents' genes) at meiosis, independently of the possible confounding factors. It is well known in the econometrics and causal literature, and slowly being recognized in the epidemiological literature that these properties define an instrumental variable (IV)[7]

[7] Didelez, (2007), "Mendelian Randomization as an Instrumental Variable Approach to Causal Inference," *Stat Methods Med Res*.

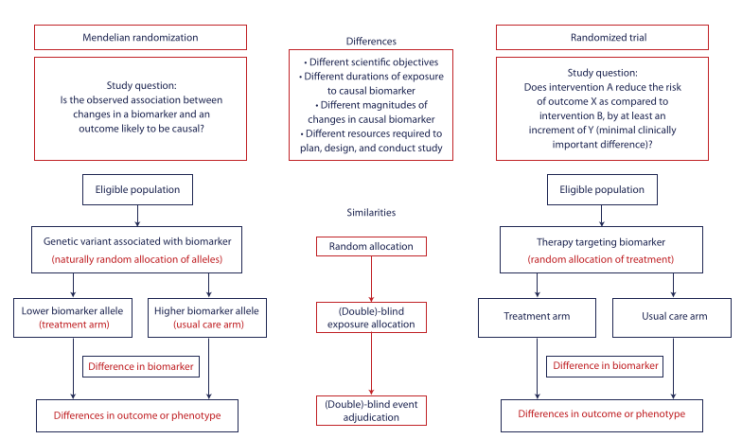
Analogy RCT - Mendelian Randomization[9]



[8] Minica Camelia, "Mendelian Randomization"; Costet, (2008), "PCSK9 and LDL Cholesterol," *Trends Biochem Sci*.

[9] Minica Camelia, "Mendelian Randomization"; Costet, (2008), "PCSK9 and LDL Cholesterol," *Trends Biochem Sci*.

Similarities MR - RCT



10

[10]Ference, (2021), "Using Mendelian Randomization to Improve the Design of Randomized Trials," *Cold Spring Harb Perspect Med*.

Similarities MR - RCT[12]

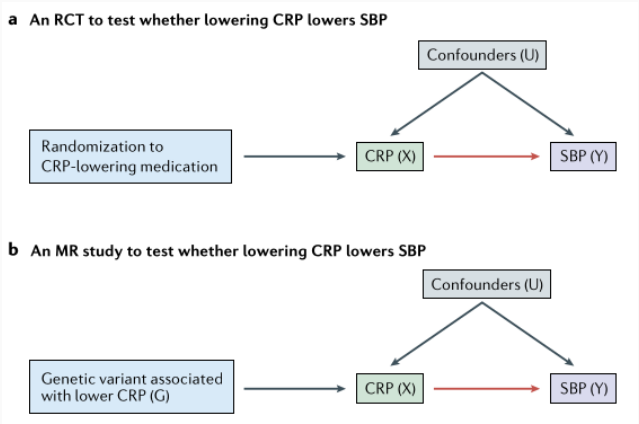


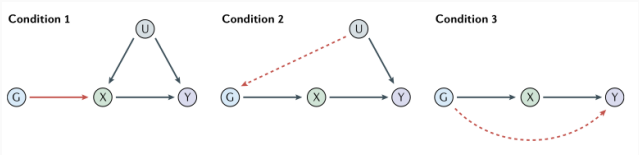
Figure 3: Illustration of a randomized control study and instrumental variable estimation. A randomized controlled trial (RCT) (panel a) and a Mendelian randomization (MR) study (panel b) to estimate the effect of lowering C-reactive protein (CRP) on systolic blood pressure (SBP). The arrows highlighted in red show the causal effect of interest.

[11]Sanderson, (2022), "Mendelian Randomization | Nature Reviews Methods Primers," *Nature Reviews Methods Primers*.

[12]Sanderson, (2022), "Mendelian Randomization | Nature Reviews Methods Primers," *Nature Reviews Methods Primers*.

MENDELIAN RANDOMIZATION

- Uses genetic variants as instrumental variables[13]
- Helps understand causation, but has strong assumptions[14]
  1. G (instrument) is robustly associated with X (“relevance”);
  2. G does not share common causes (C) with Y (Outcome) (“independence” or “exchangeability”); and
  3. G affects Y exclusively through its effect on X (“exclusion restriction”).
  4. No bidirectional causation between X and Y



[13]Richmond, (2022), “Mendelian Randomization,” *Cold Spring Harb Perspect Med*.

[14]Eleanor Sanderson, M. Maria Glymour, Michael V. Holmes, Hyunseung Kang, Jean Morrison, Marcus R. Munafò, Tom Palmer, C. Mary Schooling, Chris Wallace, Qingyuan Zhao, and George Davey Smith, “Mendelian Randomization | Nature Reviews Methods Primers.”

- Depends on intruments sufficiently predictive of exposure[15]
- Psychiatric disorders polygenicity, weak instrument bias
- Cause is (typically) assessed in one direction

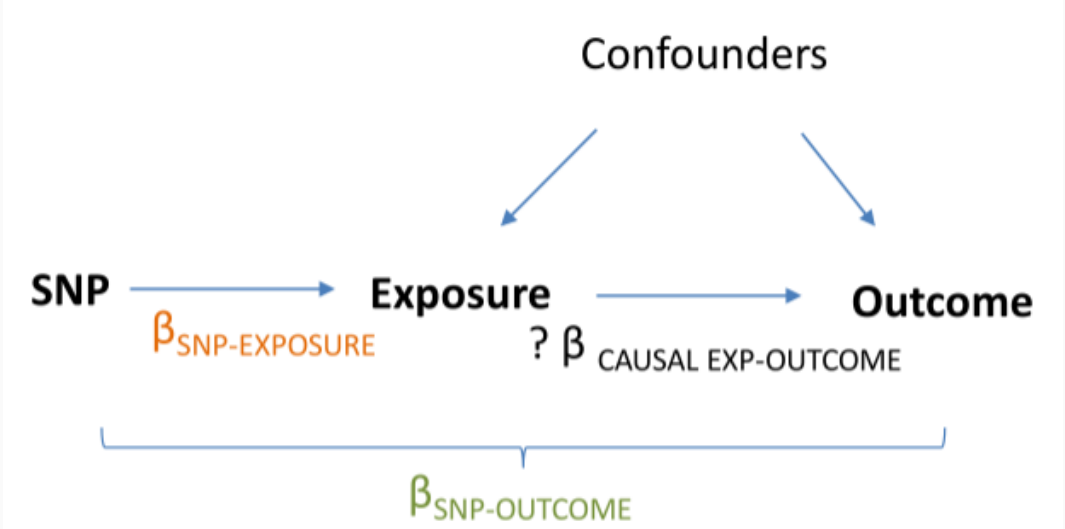
Table 4. Extensions to the basic Mendelian randomization (MR) approach

Method	Description	Directed acyclic graphs (DAGs)	Applications
Bidirectional or reciprocal MR (Timpson et al. 2011)	Used to evaluate the causal direction(s) of effect between two traits X and Y, with the use of valid instruments G_X and G_Y	$G_1 \rightarrow X \rightarrow Y$ $G_2 \rightarrow Y \rightarrow X$	Body mass index (BMI) and vitamin D (Vimaleswaran et al. 2013)

[15]Rebecca C. Richmond and George Davey Smith, "Mendelian Randomization."

Why are genetic associations special?

- Robustness to confounding due to Mendel's laws:
  - Law of segregation: inheritance of an allele is random and independent of environment etc
  - Law of independent assortment: genes for different traits segregate independently (assuming not in LD)
- The direction of causality is known – always from SNP to trait
- Genetic variants are **potentially** very good instrumental variables
- Using genetic variants as IVs is a special case of IV analysis, known as Mendelian randomization


Confounders
SNP
Exposure
Outcome
βSNP-EXPOSURE
? β CAUSAL EXP-OUTCOME
βSNP-OUTCOME

Two-stage least squares (2SLS)

- Stage 1: regress exposure on instrument and get predicted values
- Stage 2: use predicted exposure to predict the outcome

Two-stage least squares (2SLS)[17]

Stage 1

$$X_i = \beta_0 + \beta_1 * GV_i + \epsilon_i$$

- Regress exposure on GV & obtain **predicted** values

Stage 2

$$Y_i = g_0 + g_1 * \hat{X}_i + \epsilon_i$$

- Regress outcome on predicted exposure

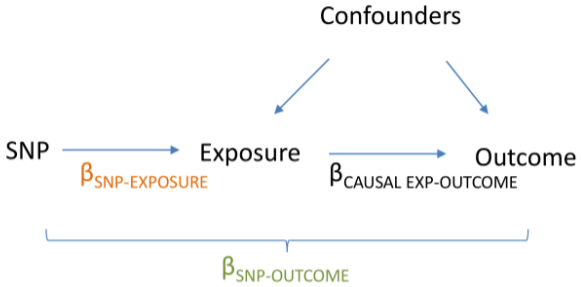
g_1 difference in outcome per unit change in (genetically-predicted) exposure

- needs to be done in one sample

[16] Minica Camelia, "Mendelian Randomization."

[17] Minica Camelia, "Mendelian Randomization."

The Wald estimator



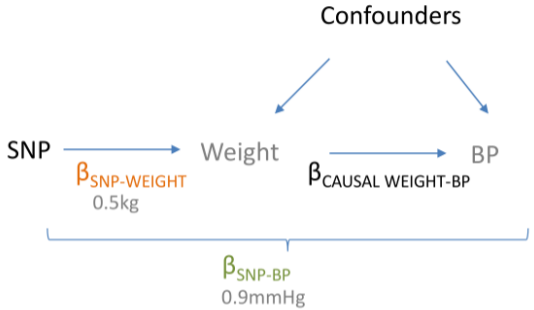
Causal effect by Wald Estimator* :

$$\frac{\hat{\beta}_{SNP\text{-}OUTCOME}}{\hat{\beta}_{SNP\text{-}EXPOSURE}}$$

$$\beta_{SNP\text{-}OUTCOME} = \beta_{CAUSAL\ EXP\text{-}OUTCOME} \times \beta_{SNP\text{-}EXPOSURE}$$

- can be used in different samples

The Wald estimator



Causal effect by Wald Estimator* :

$$\frac{\hat{\beta}_{SNP\text{-}OUTCOME}}{\hat{\beta}_{SNP\text{-}EXPOSURE}}$$

= change in outcome per unit change in exposure

BP and weight:

$$\frac{0.9\ \text{mmHg/allele}}{0.5\ \text{kg/allele}}$$

=1.8 mmHg/kg

MR CAN ALSO BE PERFORMED USING JUST THE RESULTS FROM GWAS

- Also known as two-sample MR, SMR, or MR with summary data etc
- Advantages:
  - The data is readily available, non-disclosive, free, open source
  - The exposure and outcome might not be measured in the same sample
  - The sample size of the outcome variable, key to statistical power, is not limited by requiring overlapping measures of the exposure
- Disadvantages:
  - Some extensions of MR not possible, e.g. non-linear MR, use of GxE for negative controls, various sensitivity analyses

One-sample vs Two-sample MR designs

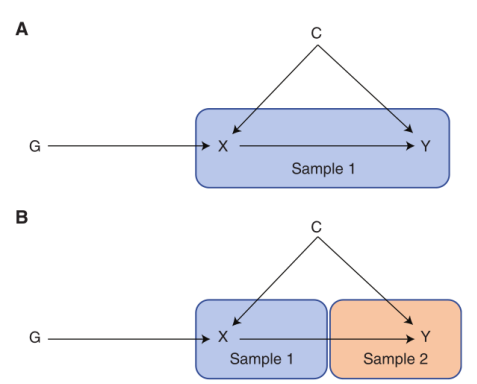


Figure 4: (A) One-sample MR uses a data set in which genotype, exposure, and outcome have been assessed. (B) Two-sample MR uses a genetic data set in which the exposure has been measured (to derive SNP-exposure estimates, sample 1) and a second genetic data set in which the outcome has been measured (to derive SNP-outcome estimates, sample 2).

18

[18]Rebecca C. Richmond and George Davey Smith, "Mendelian Randomization."

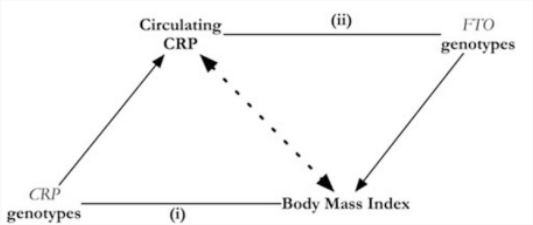
An Example using Mendelian randomization

MR Example using CRP

- C-Reactive Protein (CRP) is a biomarker of inflammation
- It is associated with BMI, metabolic syndrome, CHD and a number of other diseases
- It is unclear whether these observational relationships are causal or due to confounding or reverse causality
- This question is important from the perspective of intervention and drug development

Bi-directional MR

- Only achieved by running twice[a]



[a] Timpson, (2011), "C-Reactive Protein Levels and Body Mass Index," *Int J Obes (Lond)*.

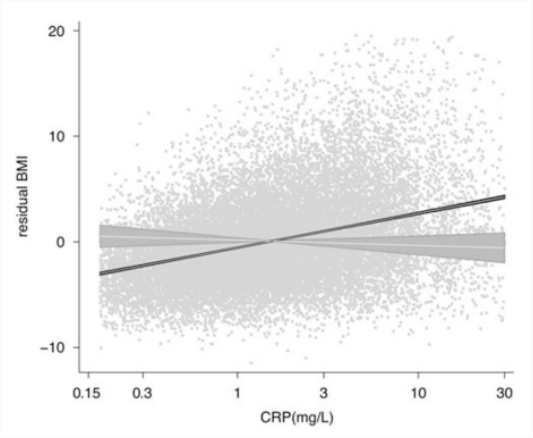
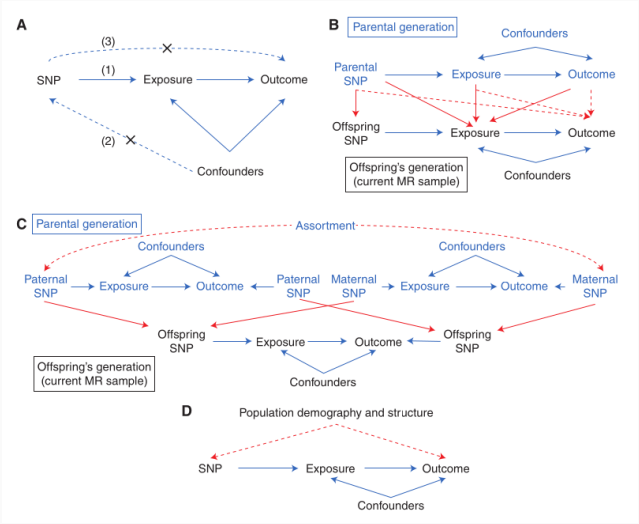


Figure 5: Comparison of linear relationships between residual BMI and circulating CRP observationally and when estimated using the CRP locus rs3091244 as an instrument for log-transformed CRP

Limitations to Mendelian randomization

1. Population stratification
2. The existence of instruments
3. Power and “weak instrument bias”
4. Pleiotropy

Dynastic effect, assortative mating and pop structure



The solid blue arrows represent relationships within an individual (e.g., the effects of offspring single-nucleotide polymorphisms [SNPs] on offspring exposure). The red lines represent the effects of relationships between individuals (e.g., the direct effects of parents' phenotypes on their children). B, dynastic effects; C, cross-trait assortative mating (educated females and tall men); D, population structure[a]

[a] Hwang, (2021), "Integrating Family-Based and Mendelian Randomization Designs," *Cold Spring Harb Perspect Med*.

Power and Weak Instruments

- Power:
  - Genetic variants explain very small amounts of phenotypic variance in a given trait
  - VERY large sample sizes are generally required
- Weak instruments:
  - Genetic variants that are weak proxies for the exposure.
  - Results in biased causal estimates from MR
- Different impact of the bias from weak instruments:
  - **Single Sample MR:** to the confounded estimate
  - **Two-Sample MR:** to the null

Using Multiple Genetic Variants as Instruments

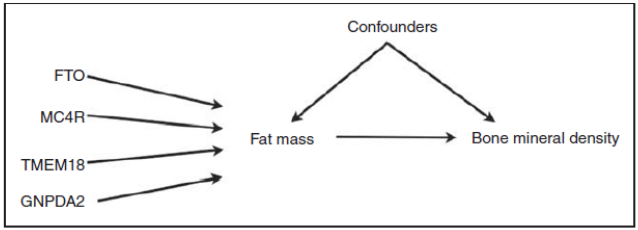
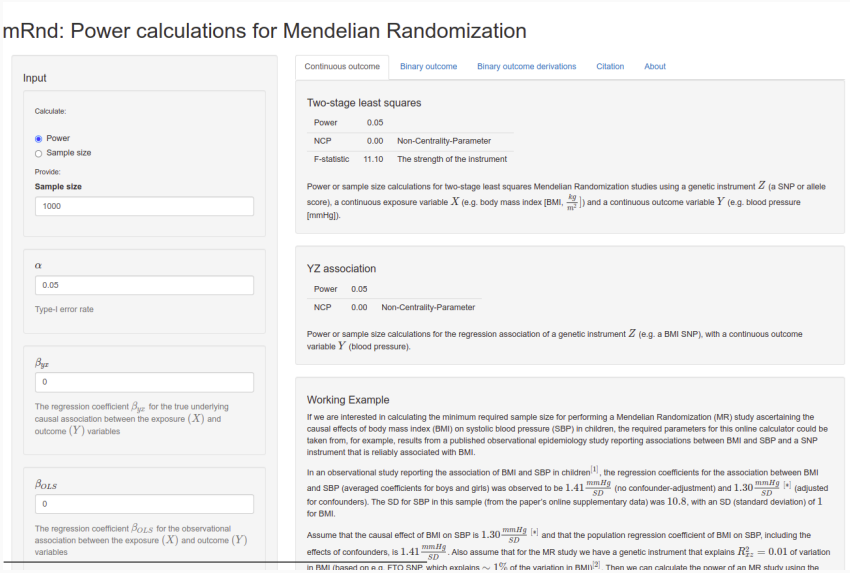


Figure 1. DAG for a Mendelian randomisation analysis using four genetic variants as instrumental variables for the effect of fat mass on bone mineral density.

- Allelic scores[19]
- Testing multiple variants individually
- Meta-analyze individual SNPs

[19]Palmer, (2012), "Using Multiple Genetic Variants as Instrumental Variables for Modifiable Risk Factors," *Stat Methods Med Res*.

Calculating Power in Mendelian Randomization Studies[21]



[20] Brion, (2013), "Calculating Statistical Power in Mendelian Randomization Studies," *Int J Epidemiol*.

Pleiotropy is pervasive

- Genetic variant influences more than one trait
- Horizontal vs Vertical pleiotropy
  - It has a central role in the genetic architecture[a]
  - Using MR-PRESSO found pleiotropy in over 48% of significant MR,[b] with large distortions on MR estimates.

[a] Jordan, (2019), "HOPS," *Genome Biology*.

[b] Verbanck, (2018), "Detection of Widespread Horizontal Pleiotropy in Causal Relationships Inferred from Mendelian Randomization Between Complex Traits and Diseases," *Nat Genet*.

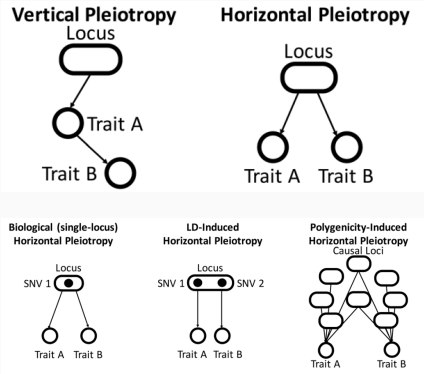


Figure 6: (LD) and polygenicity are expected to contribute to horizontal pleiotropy

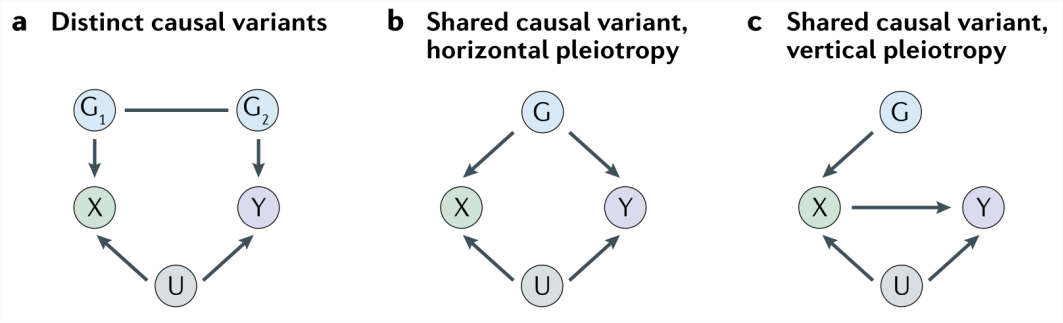


Figure 7: a | An example of distinct causal variants that violate the instrumental variable assumption IV2. G1 and G2 represent two genetic variants and the link between them is non-directional, reflecting linkage disequilibrium. b,c | Examples of a shared causal variant are a violation of assumption IV2 (panel b) and a situation that satisfies the IV assumptions (panel c).

Sensitivity analysis in MR

- Inverse variance weighted MR (unbiased estimator, but no pleiotropy assumption)
  - Heterogeneity tests
  - Multivariable MR
  - MR Egger

Inverse variance weighted (IVW) fixed effects method

- There is one underlying ‘true’ effect
- All deviations of sample effects from the ‘true’ effect are due to chance
- For N studies, each study i contributes more to the meta-analysis if its standard error is lower

$$w_i = \frac{1}{\text{var}(\beta_i)}$$

$$\beta_{\text{pooled}} = \frac{\sum_{i=1}^{N}(w_i * \beta_i)}{\sum_{i=1}^{N}(w_i)}$$

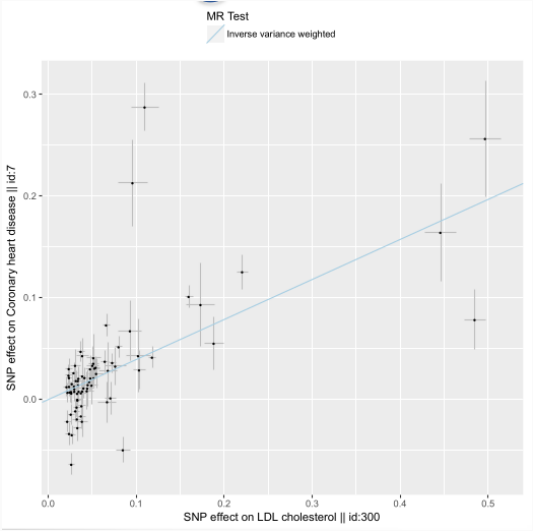
$$se_{\text{pooled}} = \sqrt{\frac{1}{\sum_{i=1}^{N}(w_i)}}$$

Calculate the p-value

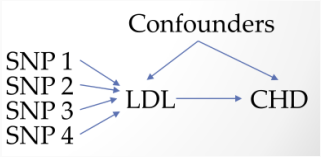
$$\chi^2_{df=1} = \frac{\beta^2_{\text{pooled}}}{se^2_{\text{pooled}}} = \frac{\left(\sum_{i=1}^{N} w_i * \beta_i\right)^2}{\sum_{i=1}^{N} w_i}$$

$$z = \frac{\beta_{\text{pooled}}}{se_{\text{pooled}}} = \frac{\sum_{i=1}^{N} w_i * \beta_i}{\sqrt{\sum_{i=1}^{N} w_i}}$$

Fixed Effects IVW-MR and Weighted Linear regression



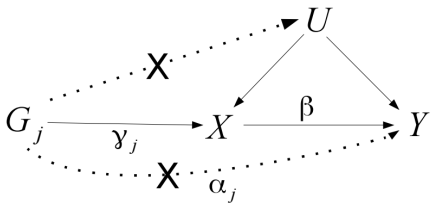
- IVW is equivalent to a weighted regression of SNP-outcome effects on SNP-exposure effects passing through the origin
- The weights are the inverse of the variance of the individual causal effect estimates
- The slope is the estimate of the causal effect



What is the problem?

- Mendelian Randomization (MR) uses genetic variants to test for causal relationships between phenotypic exposures and disease-related outcomes
- Due to the proliferation of GWAS, it is increasingly common for MR analyses to use large numbers of genetic variants
- Increased power but greater potential for **pleiotropy**
- Pleiotropic variants affect biological pathways other than the exposure under investigation and therefore can lead to biased causal estimates and false positives under the null

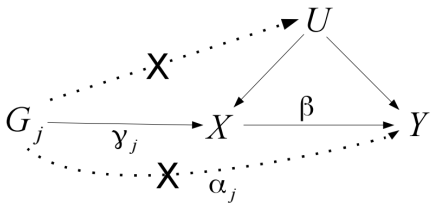
Single Variants



$$\text{Wald} = \frac{\text{Beta-GY}}{\text{Beta-GX}}$$

- Causal estimate using Wald method:
  $\frac{\beta\gamma_j}{\gamma_j} = \beta$

Multiple Variants

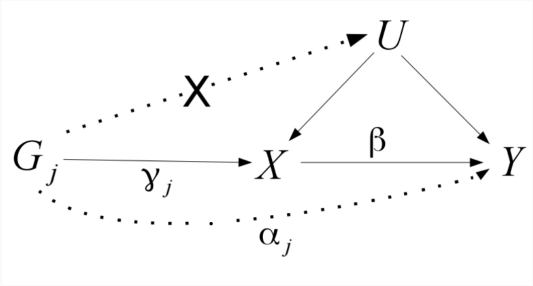


- Causal estimate using IVW from summarised data: $\frac{\sum_{j=1}^{J} \hat{\gamma}_j^2 \sigma_{Yj}^{-2} \hat{\beta}_j}{\sum_{j=1}^{J} \hat{\gamma}_j^2 \sigma_{Yj}^{-2}} = \beta$

(Approximates TSLS)

where $\hat{\beta}_j = \frac{\hat{\Gamma}_j}{\hat{\gamma}_j}$ is the ratio method estimate for variant j, and σ_{Yj} is the standard error in the regression of the outcome on the jth genetic variant, assumed to be known.

MR WITH DIRECT PLEIOTROPY



$$Y_i = \Gamma_j G_{ij} + \epsilon_{ij}^{IY}$$
$$= (\alpha_j + \beta\gamma_j)\, G_{ij} + \epsilon_{ij}^{IY}.$$

Single variant Wald estimate: $\beta_j = \beta + \frac{\alpha_j}{\gamma_j}$

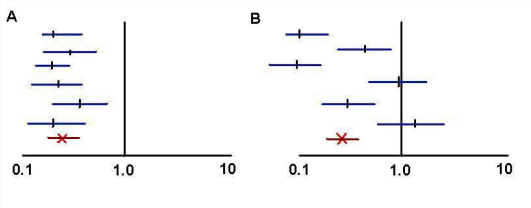
Multiple variant:

$$\beta + \frac{\sum_{j=1}^{J} \gamma_j \sigma_{Yj}^{-2} \alpha_j}{\sum_{j=1}^{J} \gamma_j^2 \sigma_{Yj}^{-2}} = \beta + \text{Bias}(\alpha, \gamma)$$

Heterogeneity

- We expect that each SNP represents an independent study, and each should give an unbiased (if imprecise) estimate of the causal effect of x on y
- Heterogeneity, where effect estimates are more different than expected due to standard errors, arises because at least some of the instruments are invalid
- Cochran's Q statistic

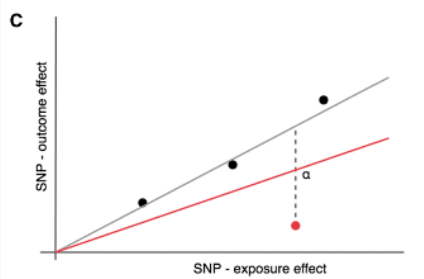
$$Q = \sum_{k=1}^{K} w_k \left(\hat{\beta}_k - \hat{\beta}_{IVW}\right)^2$$



- n=6 instruments
- Expect Q = 5 if there is no heterogeneity
- Q is chi-square distributed with n-1 degrees of freedom

Option 1: Remove outliers

- Some SNPs might contribute to the majority of the heterogeneity
- If we assume these are the invalid instruments then the IVW estimate excluding them should be less biased
- However, beware of:
  - Cherry picking, remove outliers will artificially provide a more precise estimate
  - What if the outlier is the only valid instrument, and all the others are invalid?



Option 2: Multivariable MR

- We are testing for whether X1 has an influence on Y[a]
- We know that some instruments for X1 also have influences on X2
- This opens up the possibility of horizontal pleiotropy biasing our estimate
- What is the X1-Y association adjusting for X2?

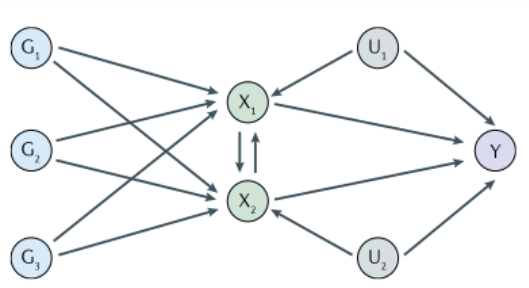


Figure 8: Multivariable Mendelian randomization (MR) for three genetic variants (G1, G2, G3), two exposures (X1, X2) and an outcome Y. Confounders U1 and U2 are assumed to be unknown.

[a] Eleanor Sanderson, M. Maria Glymour, Michael V. Holmes, Hyunseung Kang, Jean Morrison, Marcus R. Munafò, Tom Palmer, C. Mary Schooling, Chris Wallace, Qingyuan Zhao, and George Davey Smith, "Mendelian Randomization | Nature Reviews Methods Primers."

Option 3: Fit a model that is robust to horizontal pleiotropy

- IVW fixed effects estimate assumes all SNPs are valid instruments, and averages across them all
- IVW random effects model allows all SNPs to be drawn from a different distribution – the estimate is the same but the standard error is larger if there is any heterogeneity

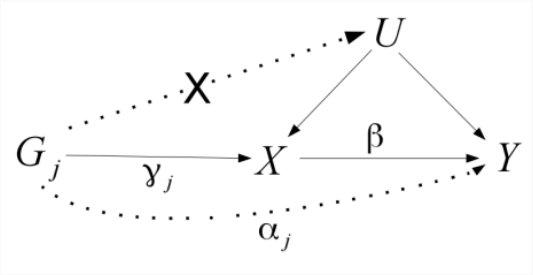
MR Egger Regression

MR Egger Regression: Central concept

- In Mendelian Randomization when multiple genetic variants are being used as IVs, Egger regression can:
  - Identify the presence of direct horizontal pleiotropy (biasing the IV estimate)
  - provide a less biased causal estimate (in the presence of pleiotropy)
- However, MR Egger lacks power

Relaxing MR's assumptions

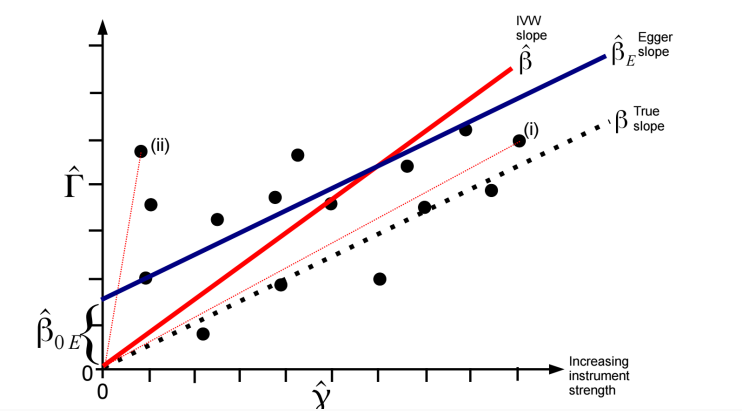
We explore the condition that the correlation between the genetic associations with the exposure (the γ_j parameters) and the direct effects of the genetic variants on the outcome (the α_j parameters) is zero. We refer to the condition that the distributions of these parameters are independent as InSIDE (Instrument Strenght Independent of Direct Effect). It can be viewed as a weaker version of the exclusion restriction assumption.



$$Y_i = \Gamma_j G_{ij} + \epsilon_{ij}^{IY}$$
$$= (\alpha_j + \beta\gamma_j)\, G_{ij} + \epsilon_{ij}^{IY}$$

All invalid instruments inside assumption satisfied

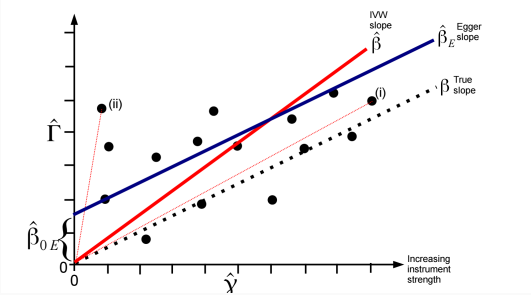
SNP – exposure association



Statistical significanc of intercept, indication of pleiotropy

- Intercept not constrained to zero

$\hat{\Gamma}_j = \beta_{0E} + \beta_E \hat{\gamma}_j$



- Egger's test assesses whether the intercept term is significantly different from zero. The estimated values of the intercept can be interpreted as the average pleiotropic effect across all genetic variants. An intercept term different from zero indicates direct pleiotropy

Summary

- IVW MR the most powerful option, but assumes the absence of horizontal genetic pleiotropy
- MR Egger, Weighted Median and Modal based estimators relax the strict requirement of no horizontal pleiotropy, but at the cost of decreased statistical power
- Crucial to perform sensitivity analyses and obtain metrics regarding the likely reliability of the MR estimates

MIXING WITH THE DOC MODEL

- We've seen:
  - Causal inference, gold standard: randomized controlled trial
  - Not always feasible, ethics[22]
    - E.g. exposure to trauma $\rightarrow$ substance abuse
- Mendelian randomization,[23] helps with these cases.
- However, pleiotropy can bias the estimates
- Now we will see that pleiotropy can be controlled for if we use a twin design
- But first we need to show proof that we can find equivalence from tsls in SEM

[22] Ohlsson, (2020), "Applying Causal Inference Methods in Psychiatric Epidemiology," *JAMA Psychiatry*.

[23] Katikireddi, (2018), "Assessing Causal Relationships Using Genetic Proxies for Exposures," *Addiction*.

Structural equation modeling - equivalence to 2SLS

- SEM solutions have been shown to recover exact estimates as 2SLS[a]
  - less convergence in weak instruments
  - slightly worse performance in ML-SEM

[a] Maydeu-Olivares, (2019), "Instrumental Variables Two-Stage Least Squares (2SLS) Vs. Maximum Likelihood Structural Equation Modeling of Causal Effects in Linear Regression Models," *Structural Equation Modeling: A Multidisciplinary Journal*.

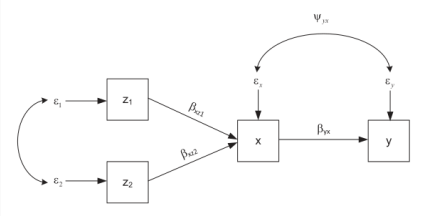


Figure 9: Instrumental Variables Regression (IVR) model that enables drawing causal inferences on the target regression model

Quick demonstration in umx()

```
library(umx)
df = umx_make_MR_data(nSubjects = 10000, Vqtl = .02, bXY= .1)

m1 = sem::tsls(formula = Y ~ X, instruments = ~ qtl, data = df)
coef(m1)
```

```
(Intercept)           X
    0.00228     0.15807
```

```
m1 = umxMR(Y~X, instruments= ~qtl, data = df)
```

	name	Estimate	SE	type
7	phi2	0.192	0.075	Factor Cov
3	eY_to_Y	1.000	0	Factor loading
4	eX_to_X	1.000	0	Factor loading
6	eY_with_eY	0.913	0.032	Factor Variance
8	eX_with_eX	0.999	0.014	Factor Variance
1	X_to_Y	0.158	0.075	Manifest path
2	qtl_to_X	0.181	0.014	Manifest path
9	one_to_X	-0.196	0.017	Mean
10	one_to_Y	0.002	0.01	Mean
11	one_to_qtl	1.007	0.007	Mean
5	qtl_with_qtl	0.494	0.007	Residual

Quick demonstration in umx()

```
df = umx_make_MR_data(10e4)

m2 <- umxRAM("myMR", data = df, autoRun = FALSE,
    umxPath(v.m. = c("qtl", "X", "Y")),
    umxPath("qtl", to = "X"),
    umxPath("X", to = "Y")
    # umxPath("X", with = "Y") # Due to OpenMx rules, will be deleted!
)
m2 <- umxModify(m2, "X_with_Y", free = TRUE, value = .2)
```

```
m1 = umxMR(Y~X, instruments= ~qtl, data = df)
```

	name	Estimate	SE	type
7	phi2	0.192	0.075	Factor Cov
3	eY_to_Y	1.000	0	Factor loading
4	eX_to_X	1.000	0	Factor loading
6	eY_with_eY	0.913	0.032	Factor Variance
8	eX_with_eX	0.999	0.014	Factor Variance
1	X_to_Y	0.158	0.075	Manifest path
2	qtl_to_X	0.181	0.014	Manifest path
9	one_to_X	-0.196	0.017	Mean
10	one_to_Y	0.002	0.01	Mean
11	one_to_qtl	1.007	0.007	Mean
5	qtl_with_qtl	0.494	0.007	Residual

Genetic variant or polygenic score?

- The use of instrumental PS is common in Mendelian randomization studies[a] .
- PS as an instrument is mathematically equivalent to a weighted mean of the results from individual SNPs.[b]

[a] Burgess, April 2020, "Guidelines for Performing Mendelian Randomization Investigations," (2020).

[b] Dudbridge, (2021), "Polygenic Mendelian Randomization," *Cold Spring Harb Perspect Med*.

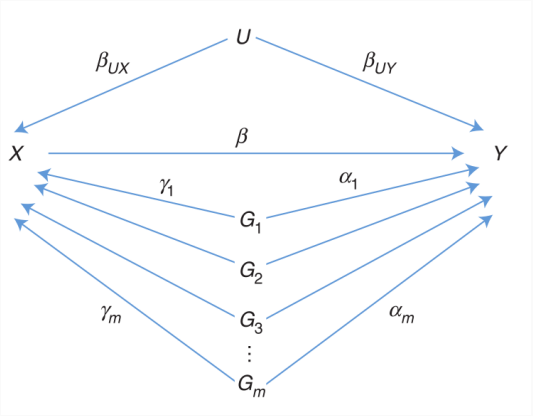
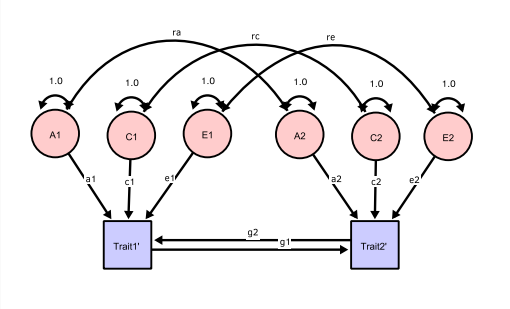


Figure 10: DAG showing m single-nucleotide polymorphisms (SNPs) Gj used as instrumental variables for the association of exposure X with outcome Y in the presence of confounder U. Each SNP has an effect yj on X and a direct pleiotropic effect alphaj on Y. Under linear models for X and Y, the total effect of SNP j on Y is shown next

Model specification



a

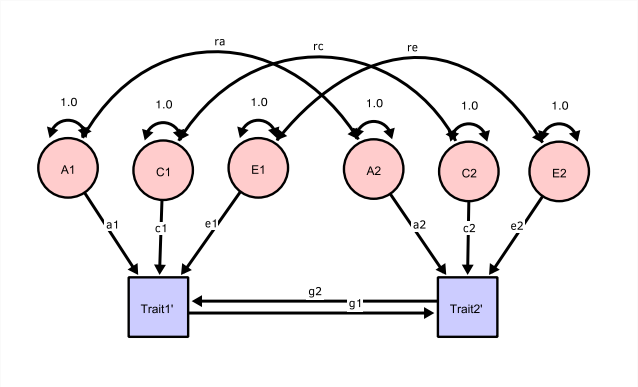
[a] Heath, (1993), "Testing Hypotheses About Direction of Causation Using Cross-Sectional Family Data," *Behav Genet*.

Path diagram representing a Bidirectional DoC for one twin.

- causal paths are estimated affording information from the cross-twin cross-trait correlations.
- Cross-twin covariance between additive genetic effects is 0.5 (not shown) for DZ twins, as DZs are expected to share 50% of the genetic effects.
- Standard SEM symbology is used.

Classic Direction of Causation

Model specification



Problems

- Not identified as depicted
- Bias at the phenotypic level[a]
- Bias due to E confounding[b]
- Better detection of cause with different variance component proportions for each phenotype[c]

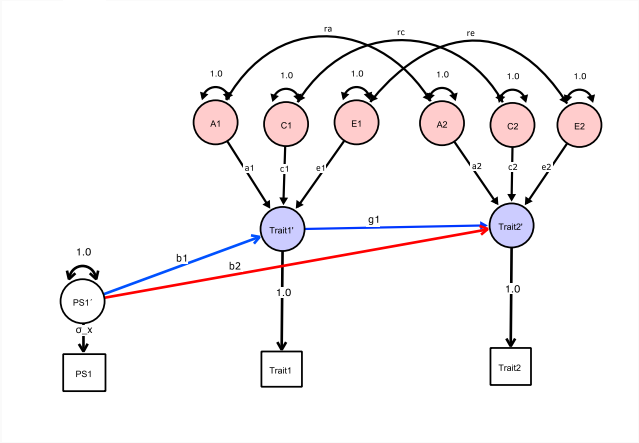
---

[a] Gillespie, (2003), "Direction of Causation Modeling Between Cross-Sectional Measures of Parenting and Psychological Distress in Female Twins," *Behav Genet*.

[b] Rasmussen, (2019), "A Major Limitation of the Direction of Causation Model," *Twin Res Hum Genet*.

[c] Maes, (2021), "Using Multimodel Inference/Model Averaging to Model Causes of Covariation Between Variables in Twins," *Behav Genet*.

MR-DoC MODEL



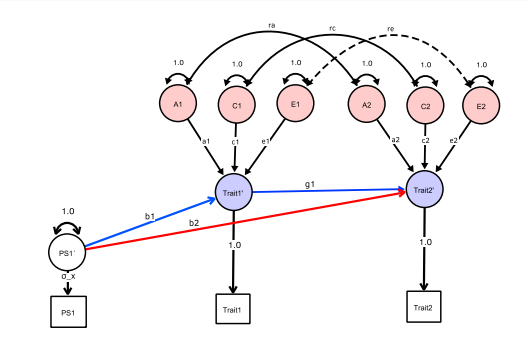
- Shown to recover exact values as a 2SLS-MR or 2sample-MR[a]
- Not identified as shown

[a] Minică, (2018), "Extending Causality Tests with Genetic Instruments," *Behav Genet*.

x	aX	cX	eX	aY	cY	eY	ra	rc	re	b1	b2	g1	Id
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	No
fr	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**fr**	**fr**	**Yes**
fr	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**Yes**
fr	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**fr**	**fr**	**fr**	**Yes**
fr	fr	0	fr	fr	0	fr	fr	0	fr	fr	fr	fr	No
fr	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**fr**	**0**	**fr**	**fr**	**fr**	**fr**	**Yes**
fr	fr	0	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	No

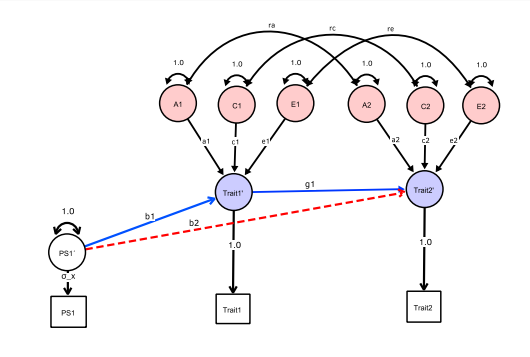
MR-DoC - IDENTIFIED CASES

x	aX	cX	eX	aY	cY	eY	ra	rc	re	b1	b2	g1	Id
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	No
fr	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**fr**	**fr**	**Yes**
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	0	fr	Yes
fr	fr	fr	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	Yes
fr	fr	0	fr	fr	0	fr	fr	0	fr	fr	fr	fr	No
fr	fr	fr	fr	fr	0	fr	fr	0	fr	fr	fr	fr	Yes
fr	fr	0	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	No



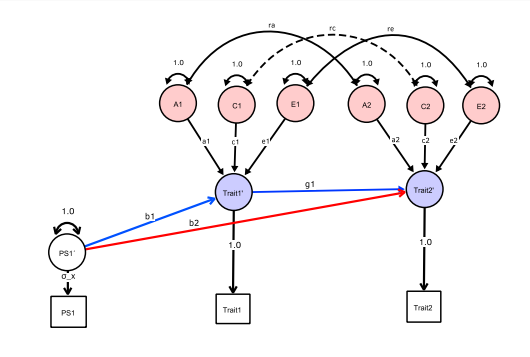
MR-DoC - IDENTIFIED CASES

x	aX	cX	eX	aY	cY	eY	ra	rc	re	b1	b2	g1	Id
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	No
fr	fr	fr	fr	fr	fr	fr	fr	fr	0	fr	fr	fr	Yes
fr	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**Yes**
fr	fr	fr	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	Yes
fr	fr	0	fr	fr	0	fr	fr	0	fr	fr	fr	fr	No
fr	fr	fr	fr	fr	0	fr	fr	0	fr	fr	fr	fr	Yes
fr	fr	0	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	No



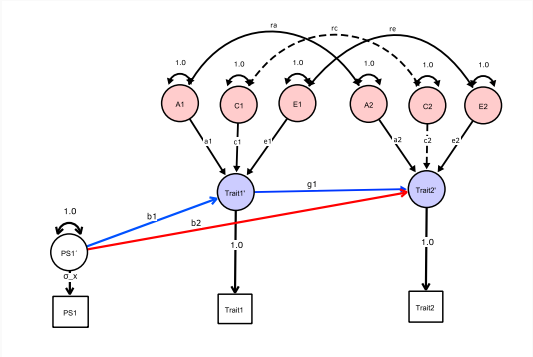
MR-DoC - IDENTIFIED CASES

x	aX	cX	eX	aY	cY	eY	ra	rc	re	b1	b2	g1	Id
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	No
fr	fr	fr	fr	fr	fr	fr	fr	fr	0	fr	fr	fr	Yes
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	0	fr	Yes
fr	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**fr**	**fr**	**fr**	**Yes**
fr	fr	0	fr	fr	0	fr	fr	0	fr	fr	fr	fr	No
fr	fr	fr	fr	fr	0	fr	fr	0	fr	fr	fr	fr	Yes
fr	fr	0	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	No



MR-DoC - identified cases

x	aX	cX	eX	aY	cY	eY	ra	rc	re	b1	b2	g1	Id
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	No
fr	fr	fr	fr	fr	fr	fr	fr	fr	0	fr	fr	fr	Yes
fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	fr	0	fr	Yes
fr	fr	fr	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	Yes
fr	fr	0	fr	fr	0	fr	fr	0	fr	fr	fr	fr	No
fr	**fr**	**fr**	**fr**	**fr**	**0**	**fr**	**fr**	**0**	**fr**	**fr**	**fr**	**fr**	**Yes**
fr	fr	0	fr	fr	fr	fr	fr	0	fr	fr	fr	fr	No



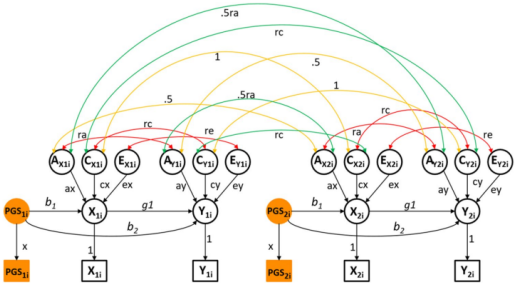


Fig. 4 Path diagrammatic representation of the MR-DoC model in DZ twins. The parameter x equals the standard deviation of the observed instrument, i.e., PGS in the circle is standardized. The model as depicted is not identified (see Table 1)

Figure 11: MR-DoC extension. Some confounding, not bidirectional. Not identified as depicted.

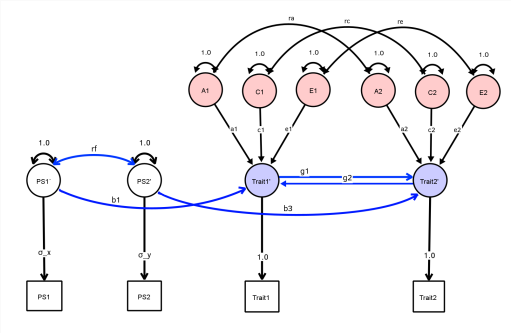
24

[24] Ibid.

Bi-directional MR-DoC, MR-DoC2

MR-DoC2[26]

Model specification



[25]Dolan, 2020, "Introducing Polygenic Risk Scores into the Twin Design," (Boulder 2020).

[26]Dolan, 2020, "Introducing Polygenic Risk Scores into the Twin Design," (Boulder 2020).

Modified MR-DOC

- Path diagram of the MR-DoC2 model for an individual.
- The model includes the effects of additive genetic (A), common environment (C) and specific environment (E) factors for both X and Y, and their effects may correlate (parameters ra, rc and re).

- Which parameters drive power?

Revisiting limitations

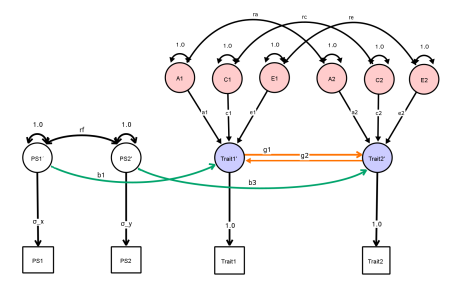
- Do we need phenotypes with distinct inheritance patterns
- What about measurement error at the phenotypic level?
- How robust it is regarding pleiotropy

WHICH PARAMETERS DRIVE POWER?[28]

Table 8: Variance explained in statistical power (non-centrality parameter; NCP) by model parameters.

	g1 = 0	g2 = 0	g1 = g2 = 0
g1	0.517	0.000	0.289
g2	0.000	0.517	0.289
b1	0.365	0.000	0.181
b3	0.000	0.365	0.181
ra	0.000	0.000	0.000
rc	0.000	0.000	0.000
re	0.002	0.002	0.000
rf	0.041	0.041	0.000
ay	0.002	0.000	0.001
ax	0.000	0.002	0.001
cy	0.002	0.000	0.001
cx	0.000	0.002	0.001
Total R2	0.929	0.929	0.945

- Distinct inheritance patterns from phenotypes - not needed
- b1, b2, g1, and g2 are what drives power
- slightly better power without C variance



[27]Van der Sluis, (2008), "Power Calculations Using Exact Data Simulation," *Behav Genet*.

[28]Van der Sluis, (2008), "Power Calculations Using Exact Data Simulation," *Behav Genet*.

Factorial design simulation - factors

θ	Design 1 (ACE)	Design 2 (AE)	Design 3 (AE)
b1	$\sqrt{0.025}, \sqrt{0.05}$	$\sqrt{0.025}, \sqrt{0.05}, \sqrt{0.075}$	$-\sqrt{0.075}, -\sqrt{0.03}, \sqrt{0.03}, \sqrt{0.075}$
b3	$\sqrt{0.025}, \sqrt{0.05}$	$\sqrt{0.025}, \sqrt{0.05}, \sqrt{0.075}$	$-\sqrt{0.075}, -\sqrt{0.03}, \sqrt{0.03}, \sqrt{0.075}$
g1	$\sqrt{0.020}, \sqrt{0.05}$	$\sqrt{0.020}, \sqrt{0.04}, \sqrt{0.06}$	$-\sqrt{0.050}, -\sqrt{0.020}, \sqrt{0.050}, \sqrt{0.020}$
g2	$\sqrt{0.020}, \sqrt{0.05}$	$\sqrt{0.020}, \sqrt{0.04}, \sqrt{0.06}$	$-\sqrt{0.050}, -\sqrt{0.020}, \sqrt{0.050}, \sqrt{0.020}$
ra	*0.25, 0.50*	*0.0, 0.25, 0.50*	0.3
rc	*0.25, 0.50*	*0*	0
re	*0.25, 0.50*	*0.0, 0.25, 0.50*	0.3
rf	*0.25, 0.50*	*0.0, 0.25, 0.50*	0.3
ax	$\sqrt{0.10}, \sqrt{0.25}$	$\sqrt{0.10}, \sqrt{0.25}$	$\sqrt{0.5}$
ay	$\sqrt{0.10}, \sqrt{0.25}$	$\sqrt{0.10}, \sqrt{0.25}$	$\sqrt{0.3}$
cx	$\sqrt{0.10}, \sqrt{0.25}$	0	0
cy	$\sqrt{0.10}, \sqrt{0.25}$	0	0
Total cells	2^{12}=4096	$3^7 * 2^2$= 8748	4^4=256

Parameter levels on the three factorial designs, with respective total number of cells for each design simulation. Also, ex was specified as as $\sqrt{1 - ax^2 - cx^2}$ and ey as $\sqrt{1 - ay^2 - cy^2}$.

- Power simulations typically involve simulating many datasets corresponding to one true model and calculating the proportion of simulations where a given effect is significant.

Exact data simulation

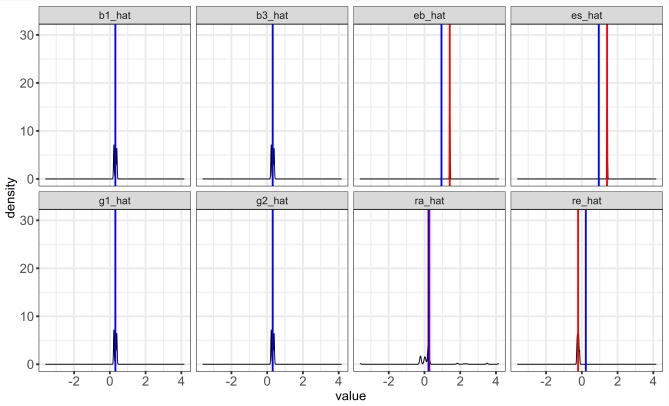
1. choosing a set of parameter values for the model shown in the figure;
2. exact data simulation, with arbitrary N=1,000 MZ pairs and N=1,000 DZ twin pairs;
3. fitting the true model using ML estimation in OpenMx;
4. fitting the false model by fixing one or more parameters to zero and refitting the model; and
5. calculating the NCP and the power to reject the false model restrictions.

- **Regressing the NCP on the parameters to work out which ones are important wrt power.**

[29] Ibid.

[30] Ibid.

Revisiting limitations: Error at the phenotypic level



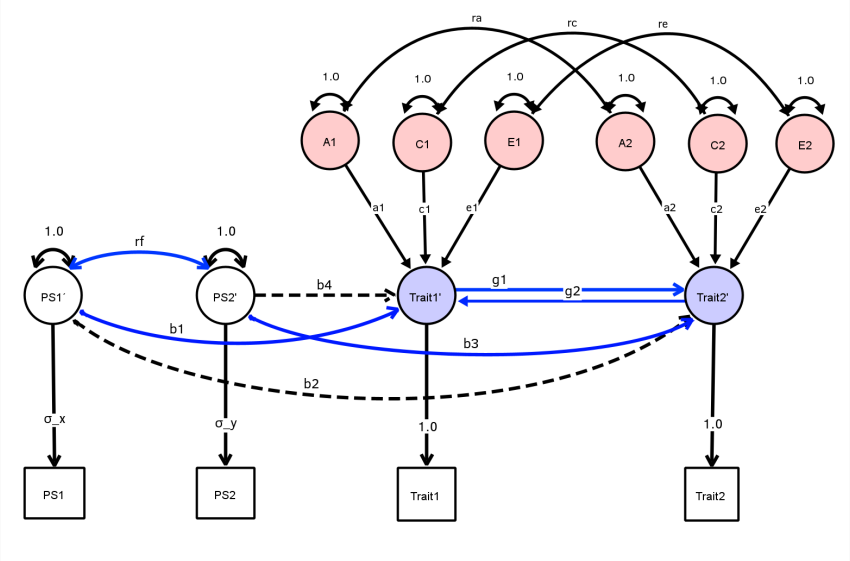
θ = {b1 = c(sqrt(.05), sqrt(.1), sqrt(.15)), b3 = c(sqrt(.05), sqrt(.1), sqrt(.15)), g1 = c(sqrt(.05), sqrt(.1), sqrt(.15)), g2 = c(sqrt(.05), sqrt(.1), sqrt(.15)), abs= .05, ass = 0.05, cbs=0.05, css=0.05, ra=.224, rc=.224, re=.224, rf=.224, reliability =c(.0, .1, .2, .3, .4)

Reliability

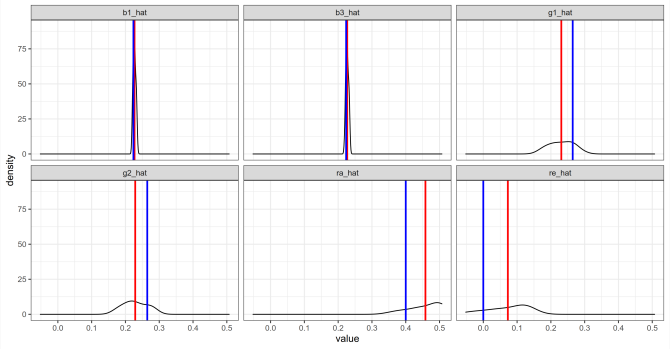
```
relB = var(B) / {var(B) + var(errorB)}
relS = var(S) / {var(S) + var(errorS)}
```

- power reduces
- but no bias in estimation of b1, b3, g1, g2

OTHER TYPES OF PLEIOTROPY NOT INCLUDED IN THE MODEL



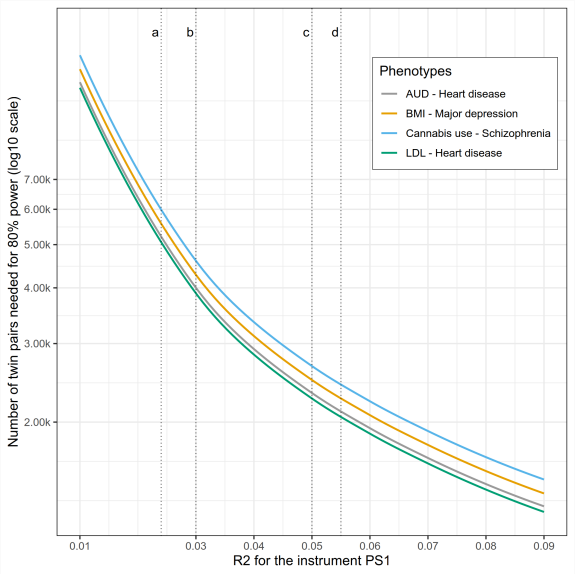
Not robust to pleiotropy (b2 and b4 !=0)



The red lines indicate the observed mean of the distribution of the estimated v while the blue lines indicated the simulated value for the parameter. If the red line is on the right of the blue line, then the parameter is overestimated and if the red line is on the left of the blue line the parameter is underestimated.

Results from simulation with 1024 replications, with variation on the factor level of b2 and b4 (0, $\sqrt{.001}$, $\sqrt{.002}$, $\sqrt{.003}$, $\sqrt{.004}$).

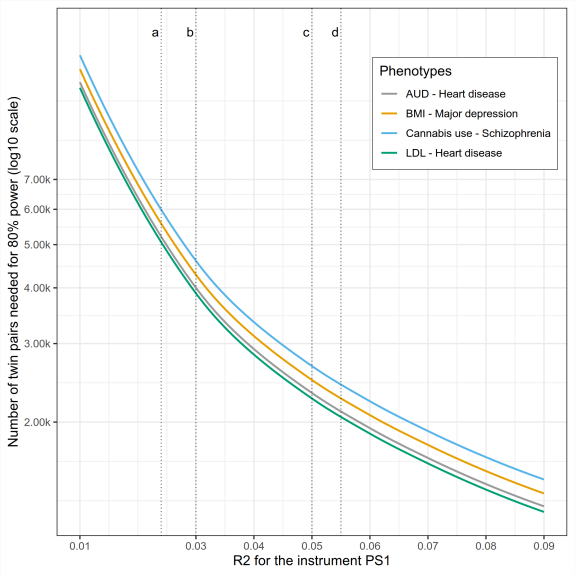
Reasonable sample sizes in relevant scenarios



A and C variances for the groups:

1. alcohol use (a^2 49%, c^2 10%) (Verhulst et al., 2015) and heart disease (a^2 22%, c^2 0%) (Wu et al., 2014);
2. BMI (a^2 72%, c^2 3%) (Rokholm et al., 2011) and major depression (a^2 37%, c^2 1%) (Scherrer et al., 2003);
3. cannabis use (a^2 51%, c^2 20%) (Verweij et al., 2010) and schizophrenia (a^2 81%, c^2 11%) (Sullivan et al., 2003);
4. dyslipidemia (LDL) (a^2 60%, c^2 28%) (Zhang et al., 2010) and heart disease (a2 22%, c^2 0%) (Wu et al., 2014).

Reasonable sample sizes in relevant scenarios



A and C variances for the groups:

1. alcohol use (a^2 49%, c^2 10%) (Verhulst et al., 2015) and heart disease (a^2 22%, c^2 0%) (Wu et al., 2014);
2. BMI (a^2 72%, c^2 3%) (Rokholm et al., 2011) and major depression (a^2 37%, c^2 1%) (Scherrer et al., 2003);
3. cannabis use (a^2 51%, c^2 20%) (Verweij et al., 2010) and schizophrenia (a^2 81%, c^2 11%) (Sullivan et al., 2003);
4. dyslipidemia (LDL) (a^2 60%, c^2 28%) (Zhang et al., 2010) and heart disease (a2 22%, c^2 0%) (Wu et al., 2014).

- Vertical lines were added to represent R2 for four PSs reported in recent papers: a, smoking (Pasman et al., 2022); b, BMI (Furlong and Klimentidis, 2020); c, LDL (Kuchenbaecker et al., 2019); d, attention deficit hyperactivity disorder (ADHD) (Demontis et al., 2019).

Behavior Genetics
https://doi.org/10.1007/s10519-022-10122-x

ORIGINAL RESEARCH

MR-DoC2: Bidirectional Causal Modeling with Instrumental Variables and Data from Relatives

Luis F. S. Castro-de-Araujo[1,2] · Madhurbain Singh[1] · Yi Zhou[1] · Philip Vinh[1] · Brad Verhulst[3] · Conor V. Dolan[4] · Michael C. Neale[1,4]

Received: 23 March 2022 / Accepted: 9 October 2022
© The Author(s) 2022

Abstract
Establishing causality is an essential step towards developing interventions for psychiatric disorders, substance use and many other conditions. While randomized controlled trials (RCTs) are considered the gold standard for causal inference, they are unethical in many scenarios. Mendelian randomization (MR) can be used in such cases, but importantly both RCTs and MR assume unidirectional causality. In this paper, we developed a new model, MRDoC2, that can be used to identify bidirectional causation in the presence of confounding due to both familial and non-familial sources. Our model extends the MRDoC model (Minică et al. in Behav Genet 48:337–349, https://doi.org/10.1007/s10519-018-9904-4, 2018), by simultaneously including risk scores for each trait. Furthermore, the power to detect causal effects in MRDoC2 does not require the phenotypes to have different additive genetic or shared environmental sources of variance, as is the case in the direction of causation twin model (Heath et al. in Behav Genet 23:29–50, https://doi.org/10.1007/BF01067552, 1993).

Keywords Causality · Pleiotropy · Twin design · Mendelian randomization

[31]Castro-de-Araujo, (2022), "MR-DoC2," *Behav Genet*.

[32]Castro-de-Araujo, (2022), "MR-DoC2," *Behav Genet*.

Tour on MR

- Requires fulfilling of strong assumptions
- Pleiotropy being the hardest one to fulfill

MR-DoC2

- Can complement MR within twin studies framework with a bidirectional causal model
- Improves on some limitations of classic DoC and MR

Future directions

- Apply MR-DoC2 to real data

Acknowledgements

Team

- Madhur Singh.
- Daniel Zhou.
- Philip Vinh.
- Brad Verhulst.

- Hermine Maes
- Conor V Dolan.
- Michael C Neale.
- NIH grant no R01 DA049867 and 5T32MH-020030

Contact

- THANK YOU