SSGG Short Course: A Introduction to Mendelian Randomization
Lecture 1: Introduction to Mendelian Randomization[1]

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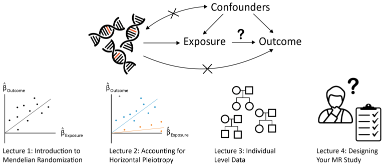

Confounders
Exposure
?
Outcome
$\hat{\beta}_{\text{Outcome}}$
$\hat{\beta}_{\text{Exposure}}$
Lecture 1: Introduction to Mendelian Randomization
$\hat{\beta}_{\text{Outcome}}$
$\hat{\beta}_{\text{Exposure}}$
Lecture 2: Accounting for Horizontal Pleiotropy
Lecture 3: Individual Level Data
?
Lecture 4: Designing Your MR Study

[1] Acknowledgment: this lecture has benefited from a short course I co-taught with Dr. Qingyuan Zhao at the UW Summer Institute.

Hierarchy of evidence

When the goal is to infer causation...

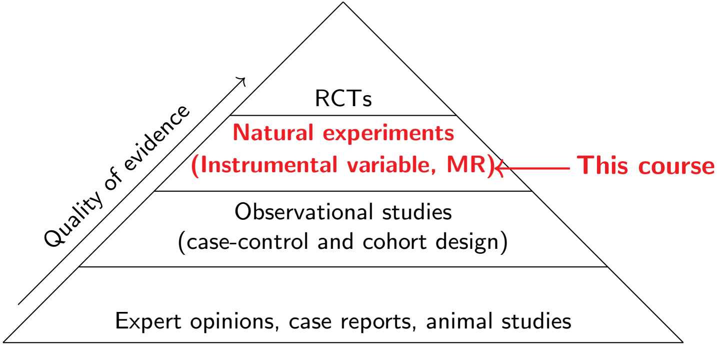
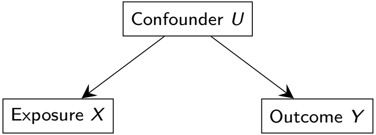

Quality of evidence
RCTs
Natural experiments
(Instrumental variable, MR)
This course
Observational studies
(case-control and cohort design)
Expert opinions, case reports, animal studies

Figure: (A rough) Hierarchy of evidence in medical studies.

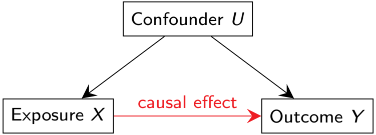
Fundamental challenges in observational studies

"Correlation does not imply causation"

- Correlation/association describes the **statistical relationship** in the data, indicating **difference in one variable is associated with difference in another**.
- Causation requires **mechanistic understanding**, indicating **intervention in one variable leads to change in another**.



(a) Correlated but not causal



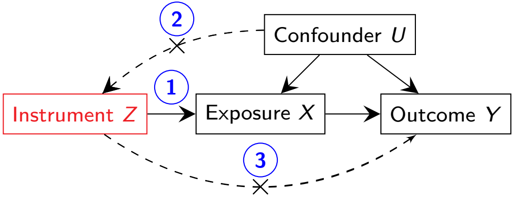
(b) Causal

Fundamental challenges in observational studies

One idea: adjusting for possible sources of spurious correlation.

- Example: Possible confounders between low density lipoprotein cholesterol (LDL-C) and coronary heart disease (CHD): age, sex, BMI, ...
- **Fundamental challenge: We can never be sure this list is complete.**
- The promise of instrumental variables: estimating causal effect without enumerating confounders.

What is an instrumental variable (IV)?

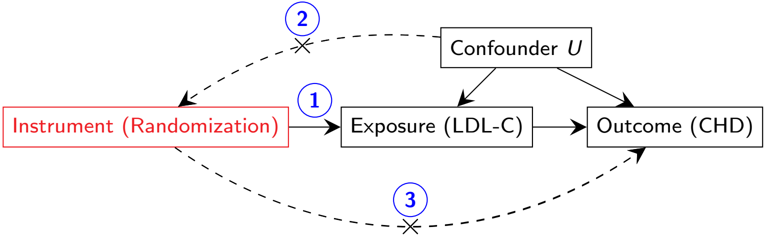


Core IV assumptions

1. **Relevance**: Z is associated with the exposure (X).
2. **Independence**: Z is independent of unmeasured confounder (U).
3. **Exclusion restriction**: Z cannot have any direct effect on the outcome (Y).

Examples of IVs

- Encouragement, physician/hospital preference, distance to care provider, calendar time, genetic variants... (Baiocchi et al., 2014)
- In an RCT, patients are randomized to take Statin for lowering LDL-C.



The Wald ratio

How it works?

- Suppose 1 unit $\uparrow$ in $Z \Rightarrow \gamma$ unit $\uparrow$ in X
- Suppose 1 unit $\uparrow$ in $X \Rightarrow \beta_0$ unit $\uparrow$ in Y (Causal effect)
- Then, 1 unit $\uparrow$ in $Z \Rightarrow$?? unit $\uparrow$ in Y

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Wald ratio: $\text{Causal effect of } X \text{ on } Y = \dfrac{\text{Effect of } Z \text{ on } Y}{\text{Effect of } Z \text{ on } X}$

What is MR?

- MR uses **genetic variants (SNPs) as IVs** to infer causation
  1. Relevance: many traits are influenced by genetics
  2. Independence: SNPs are randomly inherited from parents (Mendel's laws of inheritance)
  3. Exclusion restriction: SNPs do not have a direct effect on the outcome **(no horizontal pleiotropy) → Lecture 2**

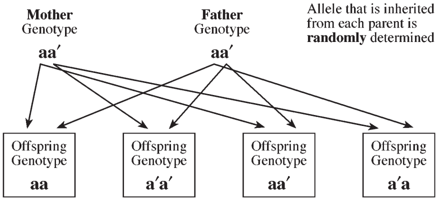


Figure 3 Mendelian randomization in parent–offspring design

Offspring should have an equal chance of receiving either of the alleles that the parents have at any particular locus

(Davey Smith and Ebrahim, 2003)

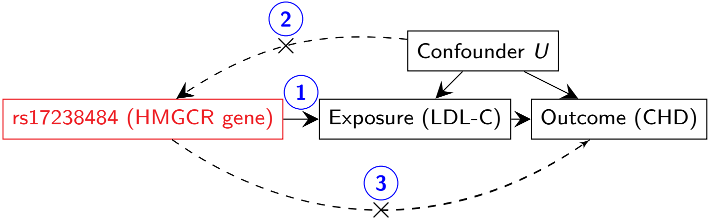
MR in non-familial studies

> *Of course populations share much common ancestry and the genetic make-up of individuals can be traced back through the random segregation of alleles during a sequence of matings, but associating genetic markers with disease risk or phenotype within such populations is not as well protected against potential distorting factors as are parent–offspring comparisons. Thus the Mendelian randomization in genetic association studies is approximate, rather than absolute.*
>
> *(Davey Smith and Ebrahim; 2003)*

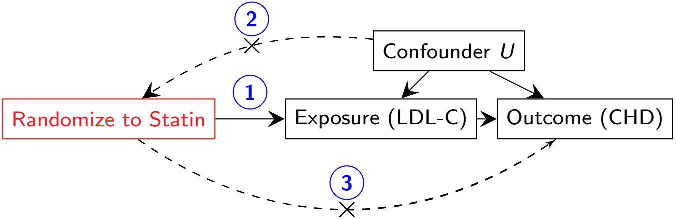
- Within-family MR → **Lecture 3**

Example: MR of LDL-C on CHD

- **Key idea**: people who inherited certain alleles tends to have higher LDL



- MR emulates a hypothetical RCT



Two-sample summary-data MR

Combine **publically available** summary data on **gene-exposure** and **gene-outcome** associations from **two separate samples**.

Zhao et al. (2020)

Two-sample summary-data MR

Combine **publically available** summary data on **gene-exposure** and **gene-outcome** associations from **two separate samples**.

Example: estimate the effect of LDL-C on CHD using $p = 160$ independent SNPs.

1. **Exposure dataset**: A GWAS for LDL-C, $\mathrm{lm}(X \sim Z_j) \Rightarrow \hat{\gamma}_j, \sigma_{Xj}, j = 1, \ldots, p$.
2. **Outcome dataset**: A GWAS for CHD, $\mathrm{lm}(Y \sim Z_j) \Rightarrow \hat{\Gamma}_j, \sigma_{Yj}, j = 1, \ldots, p$.

These two datasets are independent.

MR Assumptions

$\hat{\gamma}_j \sim N(\gamma_j, \sigma^2_{Xj}), \hat{\Gamma}_j \sim N(\Gamma_j, \sigma^2_{Yj}), j = 1, \ldots, p$, are all independent, and $\Gamma_j/\gamma_j = \beta_0$ for all j.

- Reasonable when all SNPs are independent (from LD clumping), and no overlapping sample between exposure and outcome datasets.
- For continuous outcome: β_0 is average causal effect from one unit ↑ in exposure
- For binary outcome: β_0 is a conservative causal odds ratio from one unit ↑ in exposure

Zhao et al. (2020)

Inverse-variance weighted estimator (IVW)

Wald estimator: $\hat{\beta}_j = \hat{\Gamma}_j / \hat{\gamma}_j$, with $\mathrm{var}(\hat{\beta}_j) \approx \sigma^2_{Yj} / \hat{\gamma}^2_j$.

$$\hat{\beta}_{\mathrm{IVW}} = \frac{\sum_{j=1}^{p} \hat{\gamma}_j^2 \sigma_{Yj}^{-2} \hat{\beta}_j}{\sum_{j=1}^{p} \hat{\gamma}_j^2 \sigma_{Yj}^{-2}}$$

Burgess et al. (2013)

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Burgess et al. (2013)

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- Well-known that IVW is **biased** with weak IVs.
- To mitigate the bias, usually pre-screen for strong IVs (e.g. with genome-wide significance p-value 5×10^{-8}), but using weak IVs may increase power
- **Debiased IVW** (Ye et al., 2021) uses a simple modification to effectively de-bias:

$$\hat{\beta}_{\mathrm{dIVW}} = \frac{\sum_{j=1}^p \hat{\Gamma}_j \hat{\gamma}_j \sigma_{Yj}^{-2}}{\sum_{j=1}^p (\hat{\gamma}_j^2 - \sigma_{Xj}^2) \sigma_{Yj}^{-2}}.$$

Standard error can be computed with a simple formula.

Burgess et al. (2013)

Profile likelihood method (MR-raps)

- Profile log-likelihood:

$$\ell(\beta) = -\frac{1}{2} \sum_{j=1}^{p} \frac{(\hat{\Gamma}_j - \beta\hat{\gamma}_j)^2}{\sigma_{Yj}^2 + \beta^2 \sigma_{Xj}^2}$$

- $\hat{\beta}_{\text{raps}} = \arg\max_\beta \ell(\beta)$

Zhao et al. (2020)

IV strength

- The average F-statistic is commonly used to measure IV strength:

$$\text{F-stat} = \frac{1}{p} \sum_{j=1}^{p} \frac{\hat{\gamma}_j^2}{\sigma_{Xj}^2} - 1$$

- IVW requires F-stat > 10, while dIVW requires F-stat $\cdot \sqrt{p} > 20$.

Simulations: IVW, dIVW, MR-raps

To closely mirror real applications, we take the real BMI-CAD dataset (available in the *mr.divw*) package as our simulation parameters. We use $p = 1119$ independent SNPs (pre-selected).

1. **Exposure dataset**: A GWAS for BMI in the UK BioBank ($n = 336,107$);
   $\Rightarrow \{\gamma_j, \sigma^2_{Xj}, j \in [p]\}$
2. **Outcome dataset**: A GWAS for CAD by the CARDIoGRAMplusC4D consortium ($n = 185,000$). $\Rightarrow \{\sigma^2_{Yj}, j \in [p]\}$

We set $\beta_0 = 0.4, \Gamma_j = \beta_0 \gamma_j$ (i.e., no pleiotropy). We have F-stat $= 7.8$ and F-stat $\cdot \sqrt{p} = 260.2$.

Method	mean	SD	SE	CP
IVW	0.352	0.047	0.047	82.6
dIVW	0.400	0.054	0.054	94.7
MR-raps	0.400	0.054	0.054	94.9

⟵ **Biased and poor CP**

⟵ **Unbiased and adequate CP**

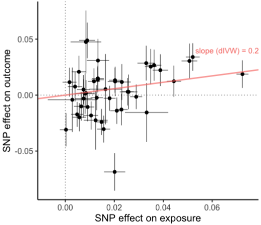
Diagnosis: tests for MR assumptions

- **F-test for weak IVs**: estimator-specific (e.g., F-stat > 10 for IVW and F-stat $> 20/\sqrt{p}$ for dIVW)
- **Modified Cochran's Q test for heterogeneity** (Bowden et al., 2019):

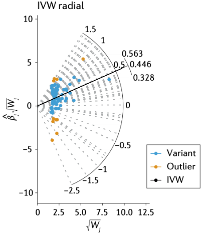
$$\text{Q statistic}: \quad Q = \sum_{j=1}^{p} \frac{(\hat{\Gamma}_j - \hat{\beta}\hat{\gamma}_j)^2}{\sigma_{Yj}^2 + \hat{\beta}^2 \sigma_{Xj}^2}$$

  If $Q > \chi^2_{1-\alpha,p-1}$, then reject H_0 : same Γ_j/γ_j across j.
- **MR-PRESSO global test, outlier test, and distortion test**:
  - Global test: $\text{RSS}_{\text{obs}} = \sum_{j=1}^{p}(\hat{\Gamma}_j - \hat{\beta}_{-j}\hat{\gamma}_j)^2$ compared against a simulated distribution under no heterogeneity
  - Outlier test: $\text{RSS}_{\text{obs},j} = (\hat{\Gamma}_j - \hat{\beta}_{-j}\hat{\gamma}_j)^2$ compared against a simulated distribution under no heterogeneity with Bonferroni correction
  - Distortion test: $D = 100 \times (\hat{\beta}_{\text{all}} - \hat{\beta}_{\text{sub}})/|\hat{\beta}_{\text{sub}}|$
- **Steiger filtering**: assess whether IVs primarily affect exposure or outcome (Hemani et al., 2017) (details → **Lecture 4**)

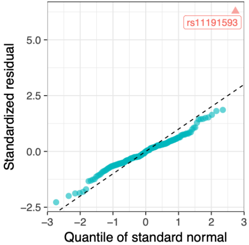
Diagnosis: visualization tools for MR assumptions



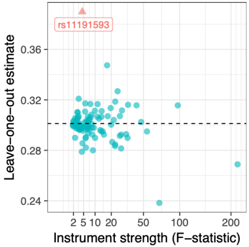
(a) Scatter plot



(b) Radial plot



(c) QQ plot

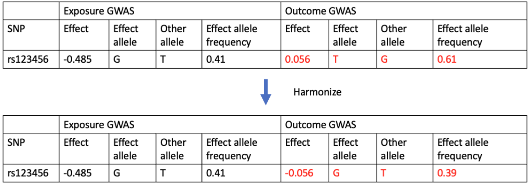


(d) Leave-one-out

Bowden et al. (2018); Zhao et al. (2020); Sanderson et al. (2022)

Basic workflow and software: BMI on CHD

1. Installation and load package: `library(TwoSampleMR)`
2. Select IVs for the exposure:
   ```
   exposure_dat <- extract_instruments("ieu-a-2", p1 = 5e-08,
                clump = TRUE, r2 = 0.001, kb = 10000)
   ```
3. Extract IVs for the outcome
   ```
   outcome_dat <- extract_outcome_data(exposure_dat$SNP, "ieu-a-7")
   ```
4. Harmonize the effect sizes
   ```
   dat <- harmonise_data(exposure_dat, outcome_dat)
   ```



	Exposure GWAS				Outcome GWAS			
SNP	Effect	Effect allele	Other allele	Effect allele frequency	Effect	Effect allele	Other allele	Effect allele frequency
rs123456	-0.485	G	T	0.41	0.056	T	G	0.61

	Exposure GWAS				Outcome GWAS			
SNP	Effect	Effect allele	Other allele	Effect allele frequency	Effect	Effect allele	Other allele	Effect allele frequency
rs123456	-0.485	G	T	0.41	-0.056	G	T	0.39

5. MR analysis and diagnosis

More complete workflow → **Lecture 4**

Practice in R (∼20min)

Strengths and challenges of MR

Strengths:

- Less susceptible to conventional unmeasured confounding
  - Mendel's laws of inheritance
- Less susceptible to reverse causation
  - Genetics are fixed at conception
- Has a summary-data and a two-sample option

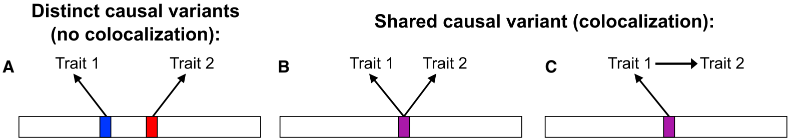
Challenges:

- Weak IV bias
- Genetic-outcome confounding
- Widespread horizontal pleiotropy can cause bias
  - Each variant has multiple biological functions
- Low power
- Assumes constant treatment effect
- Based on gene-environment equivalence
- Only applicable to heritable exposures

Song et al. (2023)

Connections to related methods

- **Colocalization** (Wallace, 2021; Zuber et al., 2022):
  - whether two traits share common genetic causal variant(s) in a gene region



  - can be used in combination with MR (Case A → violation of MR assumptions; Case B → strengthened MR conclusion)
- **Polygenic risk scores (PRS)**:
  - predicted risk for disease based on genome-wide genotypes
  - common to use PRS as an IV in MR analysis
- **Transcriptome-wide association study (TWAS)** (Gusev et al., 2016; Gamazon et al., 2015):
  - study expression-trait associations through genetic imputed expression level
  - essentially an MR of gene expression on trait (Zhu and Zhou, 2021; Zhao et al., 2024)

Summary

- MR leverages genetic variants as instruments to address causal questions
  - Emulates an RCT
  - Triangulation across multiple sources of evidence for causal inference
- MR assumptions
- Methods when all IVs are valid: IVW, dIVW, MR-raps
- Diagnosis: F-test for weak IVs, Q test and MR-PRESSO for heterogeneity, visualizations
- Basic workflow using the `TwoSampleMR` package
- Discussion: strengths and challenges, connection to other methods

Baiocchi, M., Cheng, J., and Small, D. S. (2014). Instrumental variable methods for causal inference. *Statistics in medicine*, 33(13):2297–2340.

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