Practical MR session using R

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July 22th, 2021

Outline

1. **Introduction**
    - *Two-sample MR framework*
    - *R packages for Two-sample MR*
2. **Data preparation**
    - *Summary GWAS data*
    - *Data harmonization*
3. **Performing MR analysis**
    - `MendelianRandomization` *and* `TwoSampleMR` *functions*
    - *Displaying results*
4. **Sensitivity analyses**
    - *Evaluating MR assumptions*
    - *Using MR robust methods*
5. **Case study**

Introduction

- Two sample MR analysis
- Summary instrument-exposure and instrument-outcome association results
- Summary-level data from GWAS
- Non-overlapping sets of individuals

R packages

`TwoSampleMR`

- GWAS database
- Data harmonization
- Most commonly used MR methods
- Visualization and sensitivity analyses
- Installation from Github:

```
install.packages("remotes")
remotes::install_github("MRCIEU/TwoSampleMR")
```

`MendelianRandomization`

- GWAS database
- Several MR methods are implemented
- Visualization and sensitivity analyses
- Installation from CRAN:

```
install.packages("MendelianRandomization", dependencies
```

Data sources

- Retrieving summary-level GWAS data:
  - NHGRI-EBI GWAS Catalog
  - dbGaP CHARGE Consortium GWAS results (*study accession phs000930*)
  - Neale lab
  - GeneATLAS
  - FinnGen cohort
  - Phenoscanner `→ MendelianRandomization`
  - IEU GWAS database `→ TwoSampleMR`
  - GWAS consortium websites, e.g. **CKDGen**, **GIANT**, **MAGIC**, **ENIGMA**, **ThyroidOmics**, **COVID-19 HGI**

IVs selection

- Several strategies for selecting genetic instruments

- Accounting for:
  - weak instrument bias
  - linkage disequilibrium (LD), i.e. correlations between instruments
  - horizontal pleiotropy

IVs selection

1. Selection of genome-wide significant instruments from GWAS exposure data $(p - value < 5 \times 10^{-8})$
   - One can select functional genetic variants, i.e. genome-wide significant SNPs in the encoding gene
   - Check $F - statistic$ to evaluate the strength of the instrument
2. Perform LD clumping to select independent genetic instruments
   - Choice of r^2 threshold might depend on the situation
   - $r^2 < 0.001$ is very conservative but might not work well for rare variants in small samples
   - $r^2 < 0.01$ is less conservative but acceptable for many situations
   - Some studies used more liberal thresholds, e.g. < 0.2
3. Proxies can be used to replace exposure variants that are missing in the outcome dataset
   - The proxy should be in high LD with the missing genetic instrument
4. Applying Steiger filter to remove IVs for which there might evidence of reverse causation

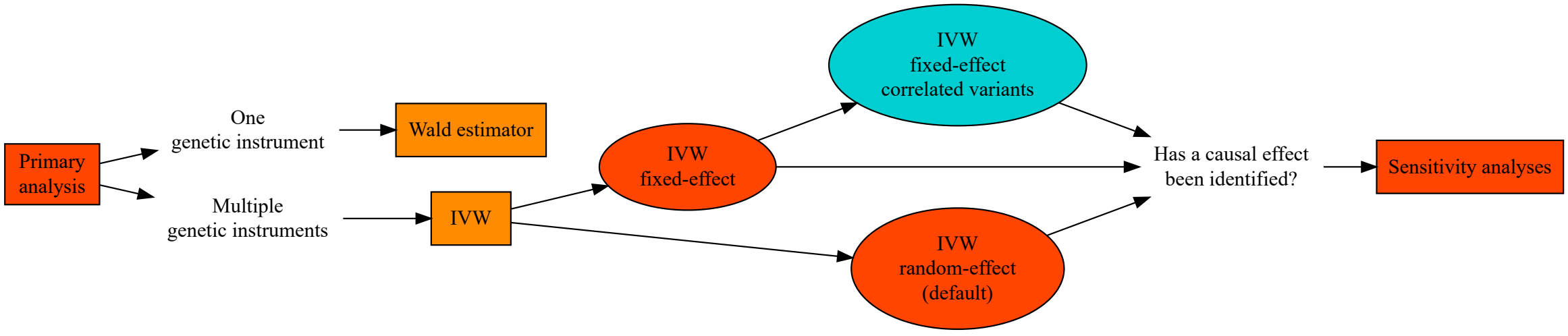
Data harmonization

Harmonise exposure and outcome effects

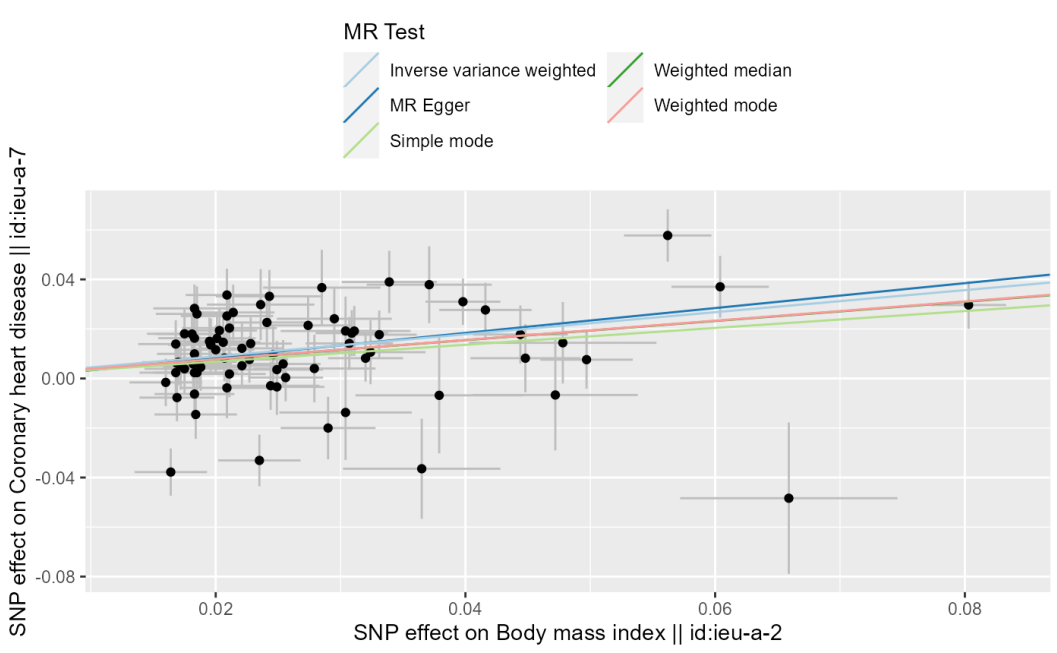
	Exposure GWAS				Outcome GWAS			
SNP	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*
rs12345	0.132	A	G	0.28	0.022	A	G	0.26
rs23456	-0.485	G	T	0.41	**0.056**	**T**	**G**	**0.61**
rs34567	0.203	G	C	0.11	**-0.046**	**G**	**C**	**0.88**

	Exposure GWAS				Outcome GWAS			
SNP	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*
rs12345	0.132	A	G	0.28	0.022	A	G	0.26
rs23456	-0.485	G	T	0.41	**-0.056**	**G**	**T**	**0.39**
rs34567	0.203	G	C	0.11	**0.046**	**G**	**C**	**0.12**

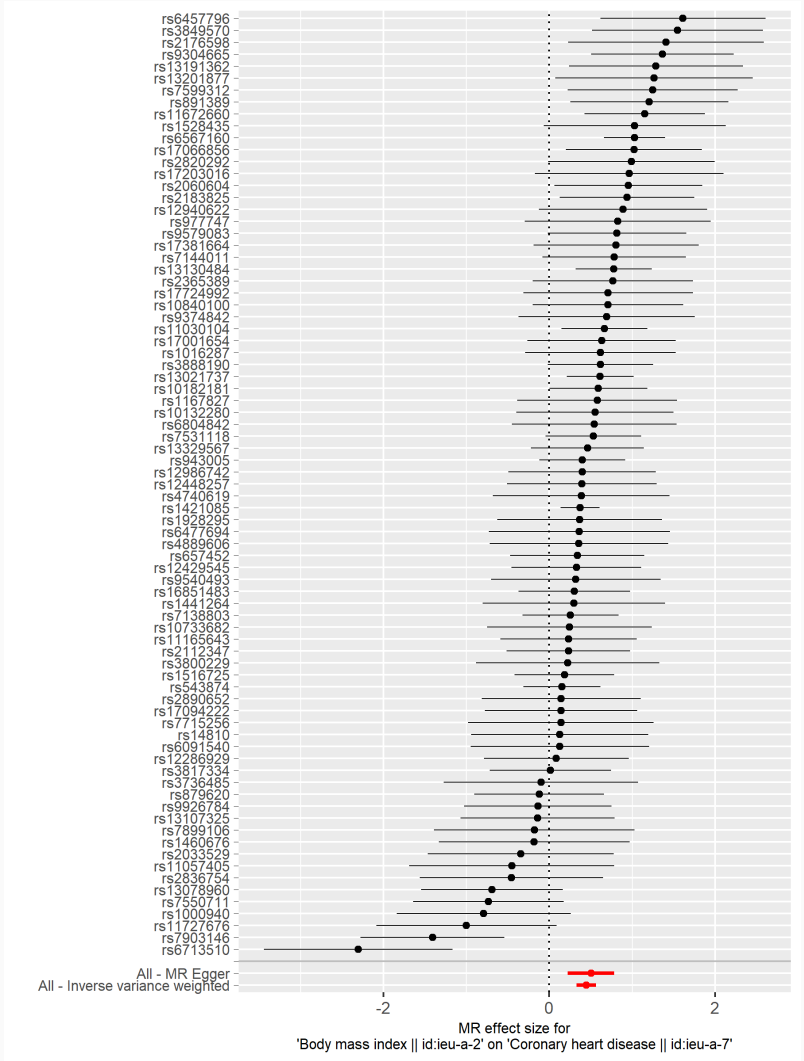
https://elifesciences.org/articles/34408


Primary analysis
One genetic instrument
Wald estimator
Multiple genetic instruments
IVW
IVW fixed-effect
IVW fixed-effect correlated variants
IVW random-effect (default)
Has a causal effect been identified?
Sensitivity analyses

MR analysis



https://mrcieu.github.io/TwoSampleMR/articles/perform_mr.html



Sensitivity analyses

- Evaluation of MR assumptions
- Check for the presence of pleiotropic effects
  - Heterogeneity of Wald estimates using Q-statistic or I^2
  - Intercept of MR-Egger
- Leave-one-out analysis to assess the reliance of MR analysis on a particular variant

Robust MR methods

- Valid causal inferences under weaker assumptions than the standard IVW methods
- Using multiple methods that make different assumptions about the nature of the underlying pleiotropy
- Most commonly used robust methods:
  - *MR-Egger* $\longrightarrow$ `TwoSampleMR` and `MendelianRandomization`
  - *Median based method* $\longrightarrow$ `TwoSampleMR` and `MendelianRandomization`
  - *Mode based method* $\longrightarrow$ `TwoSampleMR` and `MendelianRandomization`
  - *MR-PRESSO* $\longrightarrow$ `MR-PRESSO` R package
- A simulation study[1] has shown that two recent methods have good statistical properties in a wide range of scenarios:
  - *Contamination mixture* $\longrightarrow$ implemented in `MendelianRandomization` with `mr_conmix` function
  - *MR-Mix* $\longrightarrow$ `MR-Mix` R package

[1] Slob EAW, Burgess S. **A comparison of robust Mendelian randomization methods using summary data**. *Genetic Epidemiology*. 2020;44:313–29.

Other approaches to sensitivity analysis

- Using different datasets
- Positive and negative control outcomes
- Subgroup analyses
- Evaluate association with potentially pleiotropic variables

Case study

- **Aim**: to evaluate the causal relationship between kidney function and blood pressure (BP)
- **Exposure**: estimated glomerular filtration rate from serum creatinine (eGFRcr)
- **Outcome**: systolic blood pressure (SBP)

Case study

Two sets of IVs:

- 256 SNPs associated with eGFRcr at genome-wide threshold $\left(p - value < 5 \times 10^{-8}\right)$
- 40 SNPs associated with both eGFRcr and blood urea nitrogen (BUN) at genome-wide threshold $\left(p - value < 5 \times 10^{-8}\right)$ $\rightarrow$ prioritizing IVs most likely relevant for kidney function