Sensitivity Analyses in Mendelian Randomization Studies

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This Session

- Inverse variance weighted MR
- Heterogeneity tests
- Multivariable MR
- MR Egger
- MR Weighted Median
- MR Modal Estimator
- Steiger Filtering

Inverse Variance Weighted Fixed Effects Meta-analysis

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

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

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

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

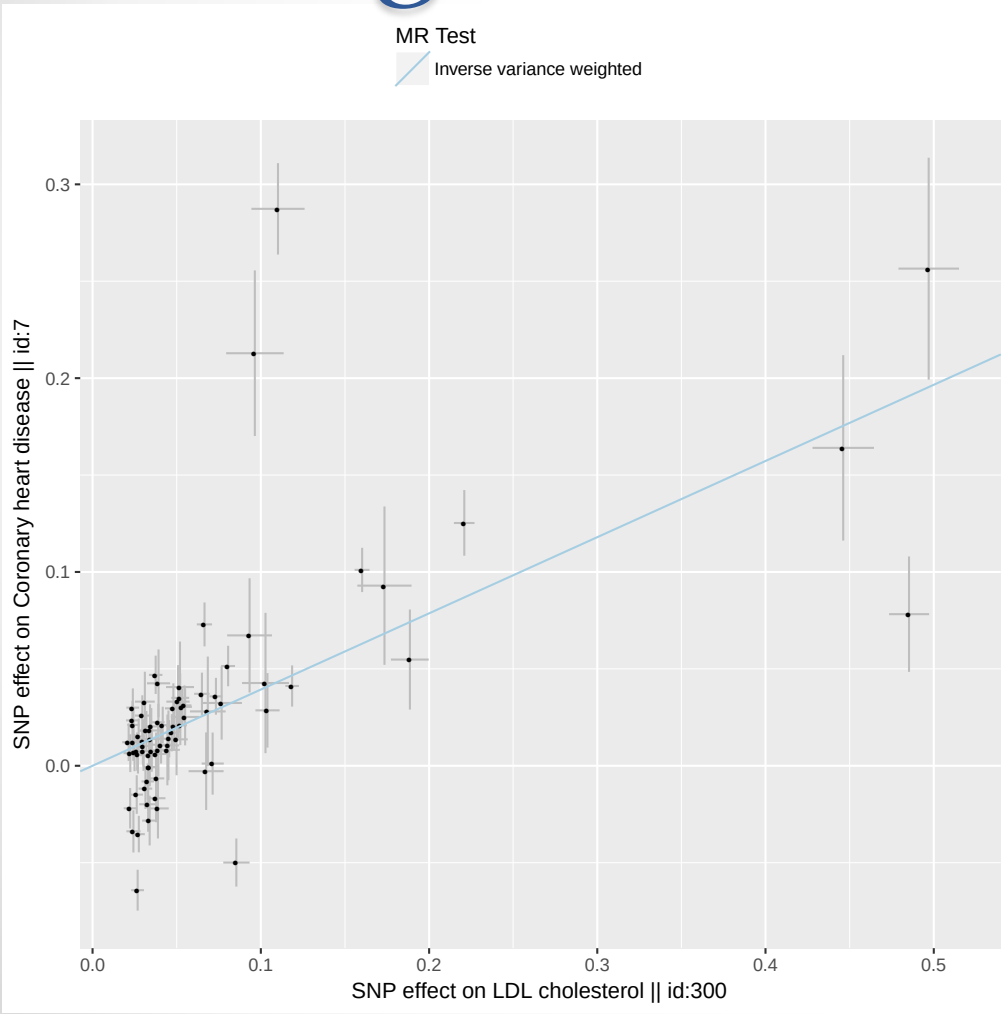
For N studies, each study *i* contributes more to the meta-analysis if its standard error is lower

Calculate p-value

$$\chi^2_{df=1} = \frac{\beta_{pooled}^{\ 2}}{se_{pooled}^{\ 2}} = \frac{(\sum_{i=1}^{N} w_i * \beta_i)^2}{\sum_{i=1}^{N} w_i}$$

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

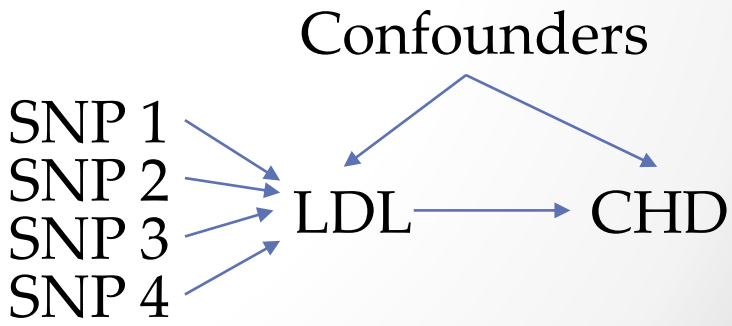
Fixed Effects IVW-MR and Weighted Linear regression



IVW is equivalent to a weighted regression of SNP-outcome effects on SNP-exposure effects passing through the origin

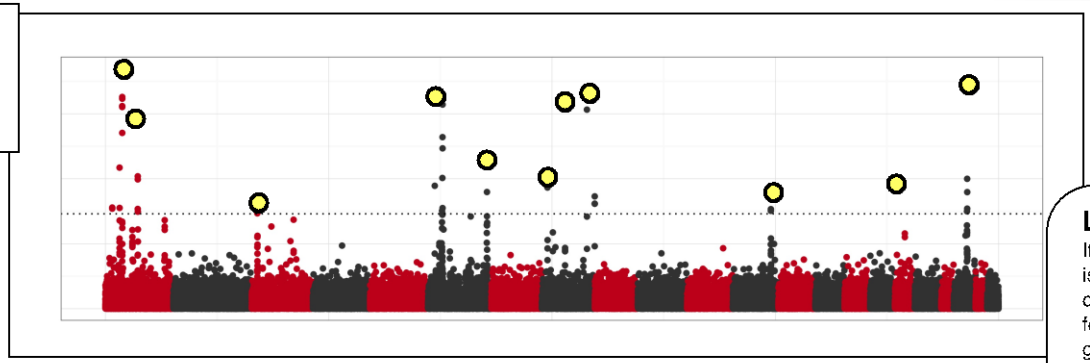
The weights are the inverse of the variance of the individual causal effect estimates

The slope is the estimate of the causal effect

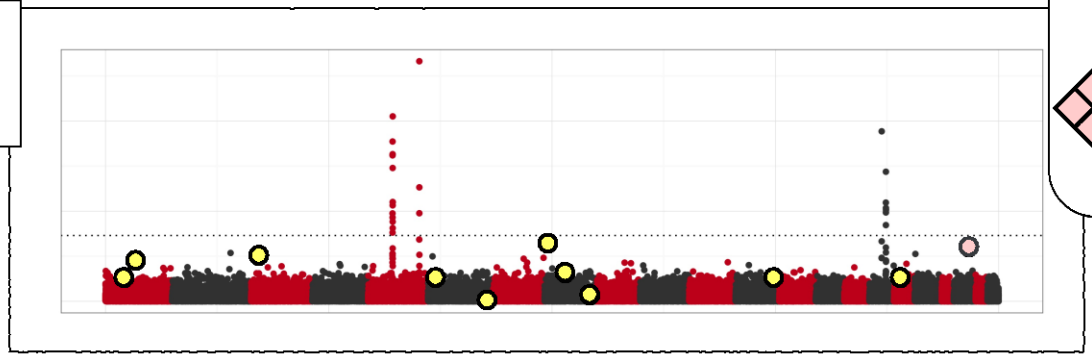


Performing MR With Summary Statistics

Obtain instruments from exposure GWAS

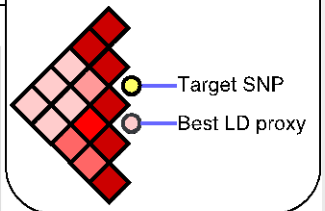


Extract SNP effects from outcome GWAS

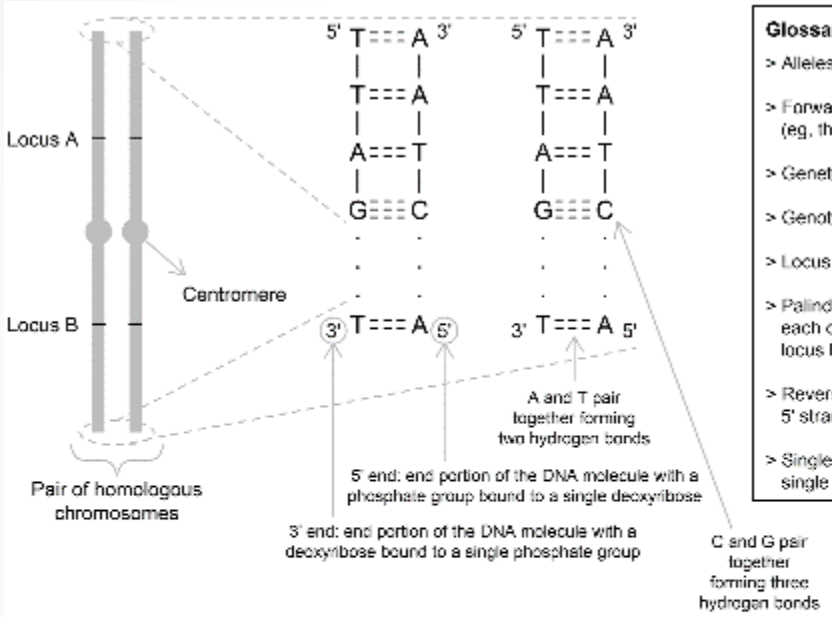


LD Proxies

If an exposure instrument is not available in the outcome GWAS then look for LD proxies in 1000 genomes



The Issue of Strand



Glossary

> Alleles: variant forms that a locus may present.

> Forward or positive strand: the DNA strand that is read from the 5' to the 3' end (eg, the 5' TTAG...T 3' strand in the figure).

> Genetic variant: locus with more than one allele in a population.

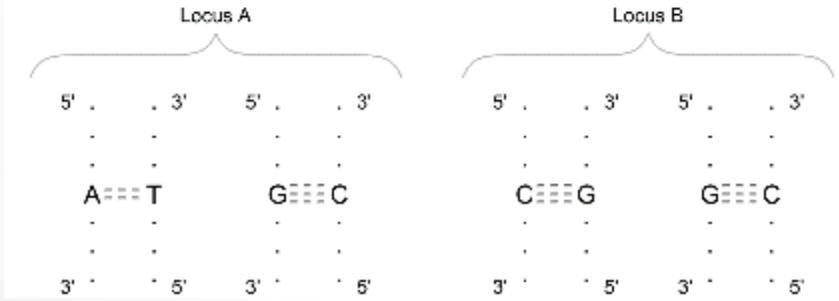
> Genotype: combination of alleles that an individual presents at a given locus.

> Locus (plural loci): a specific location in a DNA sequence.

> Palindromic SNP: SNPs whose alleles correspond to nucleotides that pair with each other in a double-stranded DNA molecule. SNPs with A/T or G/C (as in locus B below) alleles are palindromic SNPs.

> Reverse or negative strand: the DNA strand that is read from the 3' to the 5' strand (eg, the 3' AATC...A 5' strand in the figure).

> Single nucleotide polymorphism (SNP): a type of genetic variant that involves single base pair changes.

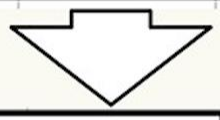


	Locus A	Locus B
Type of genetic variation	Single nucleotide polymorphism	Single nucleotide polymorphism
Alleles (5' to 3')	A and G	C and G
Alleles (3' to 5')	T and C	G and C
Genotype (5' to 3')	AG	CG
Genotype (3' to 5')	TC	GC
Palindromic variant	No	Yes

Hartwig et al 2017

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**

MR methods for handling horizontal pleiotropy

...

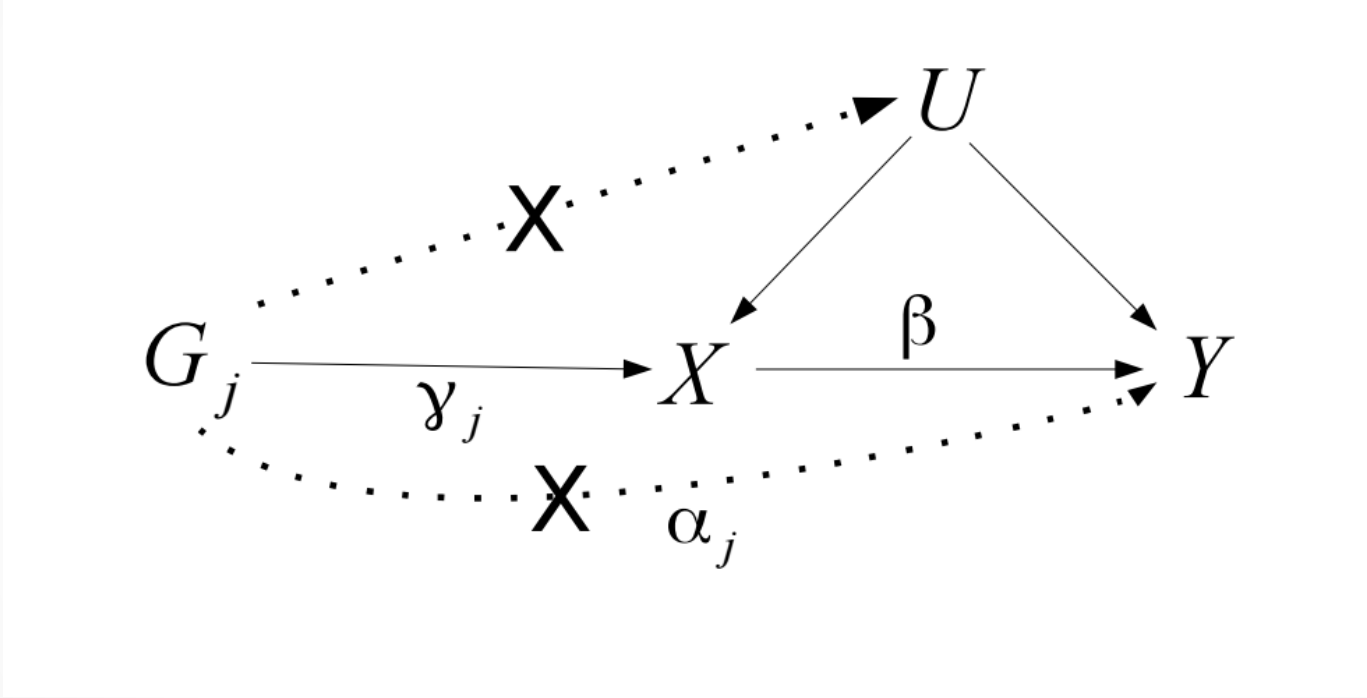
Many methods now exist

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

Two Sample MR:

Single Variants



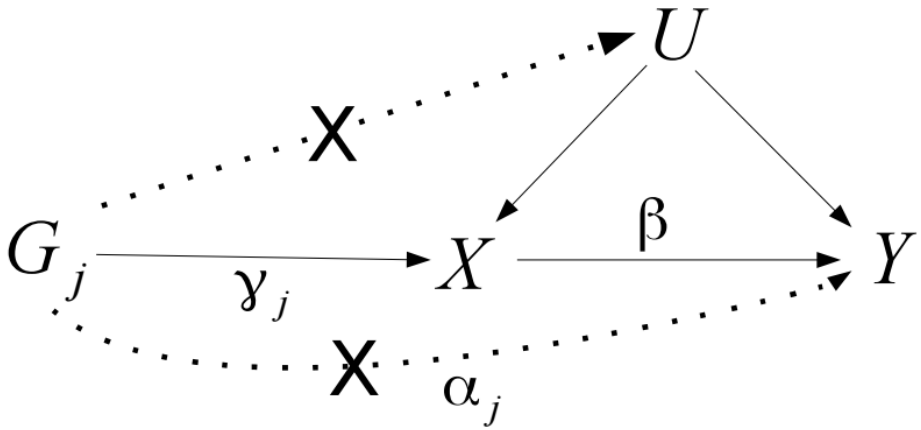
Wald = Beta-GY / Beta-GX

Causal estimate using Wald method:

$$\frac{\beta\gamma_j}{\gamma_j} = \beta.$$

Two Sample MR:

Multiple Variants



Causal estimate using IVW from summarised data:

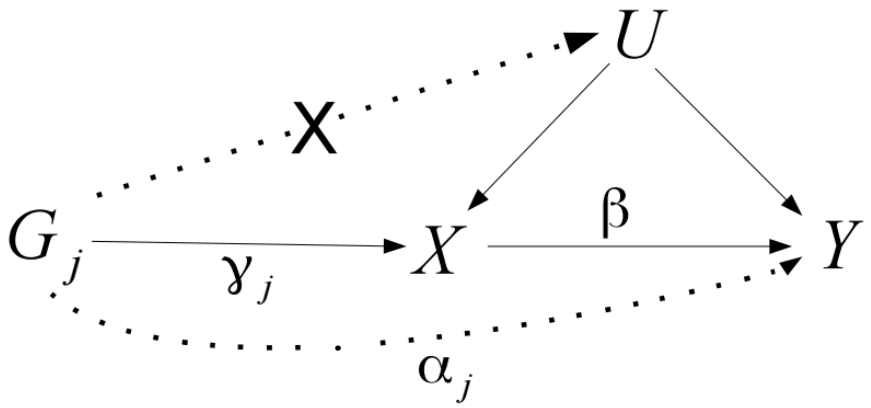
$$\frac{\sum_{j=1}^{J} \hat{\gamma}_j^2 \sigma_{Yj}^{-2} \hat{\beta}_j}{\sum_{j=1}^{J} \hat{\gamma}_j^2 \sigma_{Yj}^{-2}} = \beta.$$

(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

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



Single variant Wald estimate:

$$\beta_j = \beta + \frac{\alpha_j}{\gamma_j}$$

Multiple variant TSLS / IVW :

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

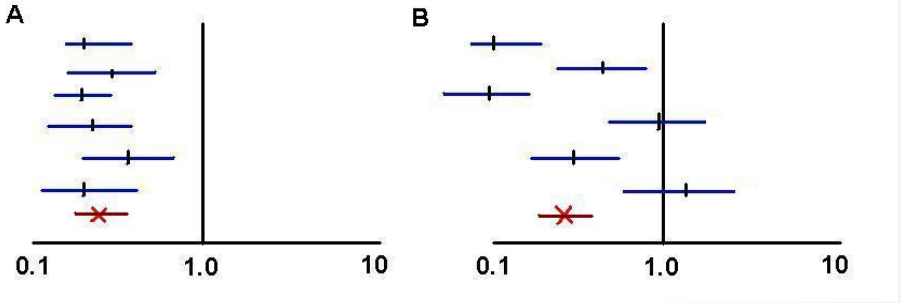
Heterogeneity

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

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



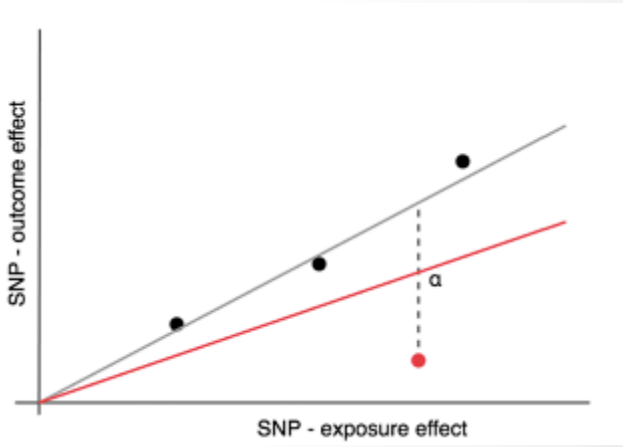
n=6 instruments
Expect Q = 5 if there is no heterogeneity
Q is chi-square distributed with n-1 degrees of freedom

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

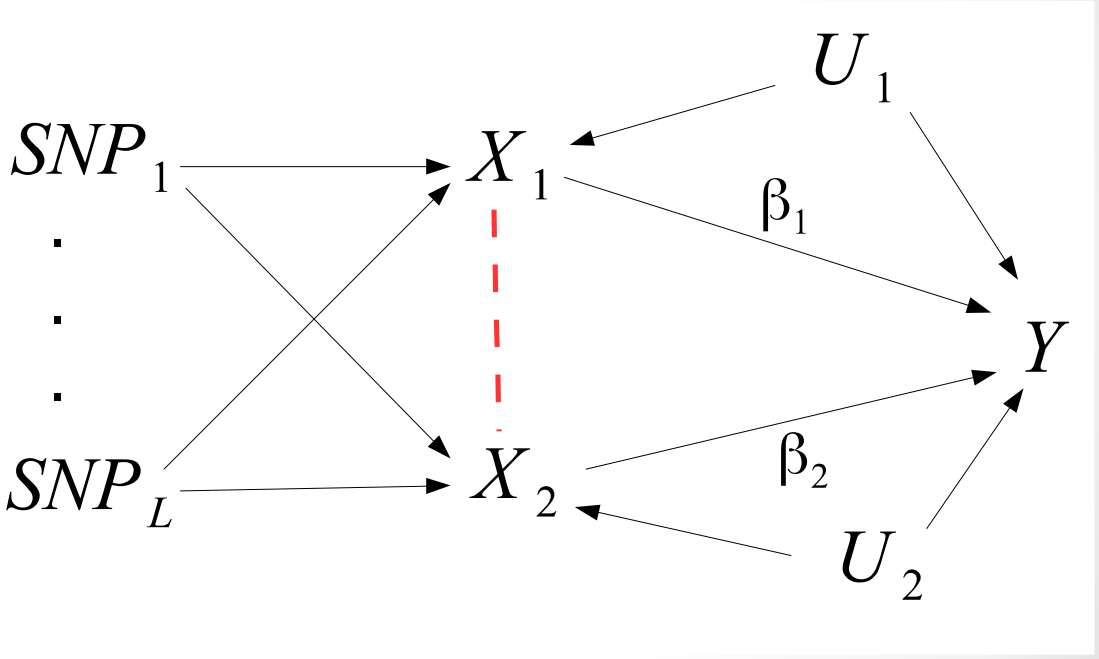
However – beware of:

- 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?
  - E.g. cis-variants for gene expression, DNA methylation, protein levels. CRP levels are best instrumented by variants within the *CRP* gene region. Most other variants that come up in CRP GWAS are upstream effects related to inflammation



Option 2: Multivariable MR

- We are testing for whether X1 has an influence on Y
- 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?



Option 3: Fit a model that is robust to some model of 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
- Several others…

MR Egger Regression

MR Egger Regression: Central concept

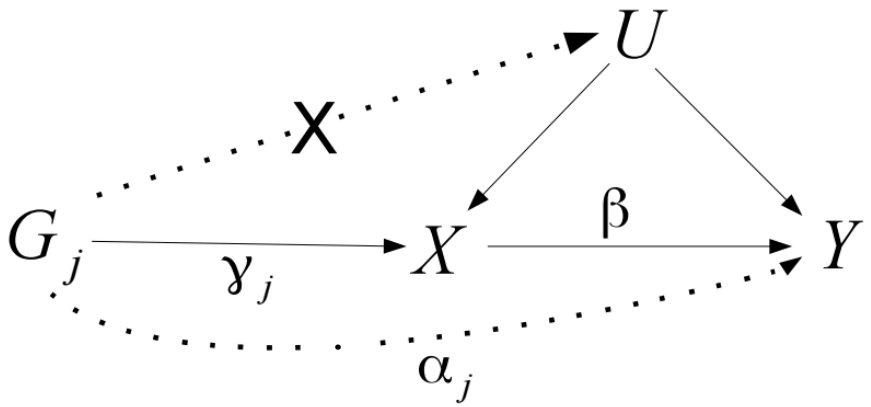
- In Mendelian Randomization when multiple genetic variants are being used as IVs, Egger regression can:
  - Identify the presence of 'directional' pleiotropy (biasing the IV estimate)
  - provide a less biased causal estimate (in the presence of pleiotropy)

However, MR Egger lacks power

InSIDE Assumption

Relaxing MR's assumptions

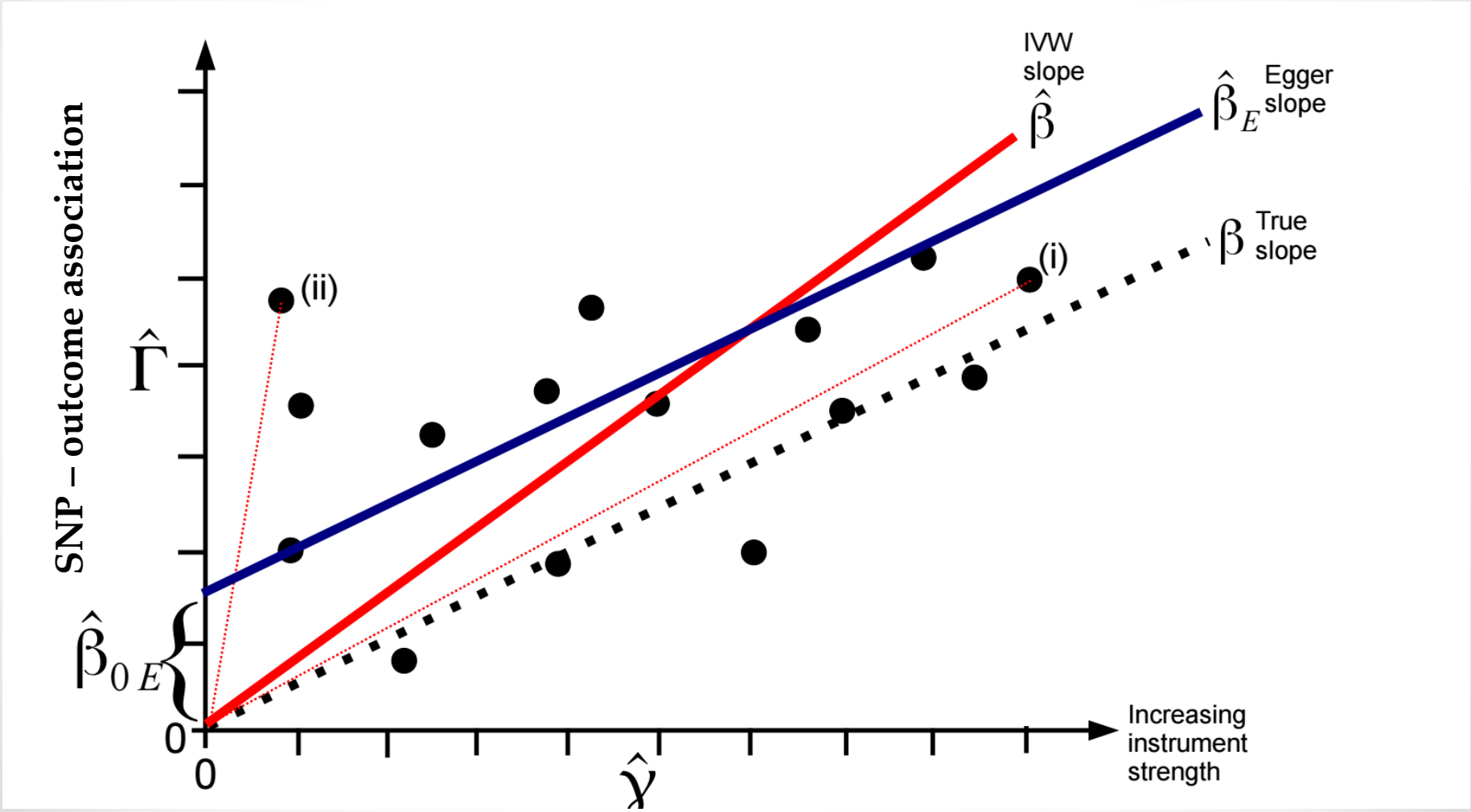
$$Y_i = \Gamma_j G_{ij} + \epsilon_{ij}^{\prime Y}$$
$$= (\alpha_j + \beta\gamma_j) G_{ij} + \epsilon_{ij}^{\prime Y}.$$



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 Strength Independent of Direct Effect). It can be viewed as a weaker version of the exclusion restriction assumption.

Example:

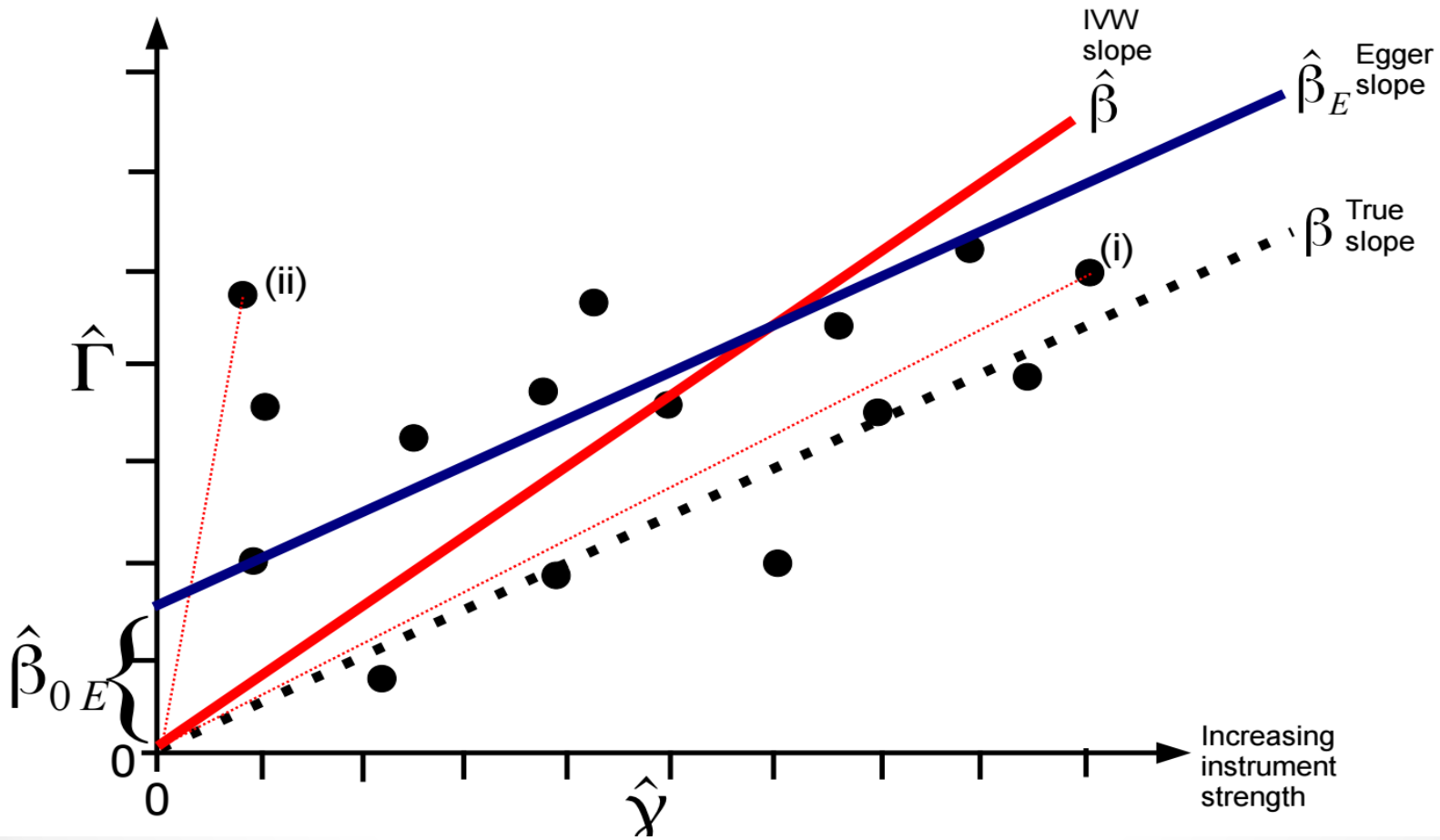
ALL INVALID INSTRUMENTS
INSIDE ASSUMPTION SATISFIED



Egger regression:

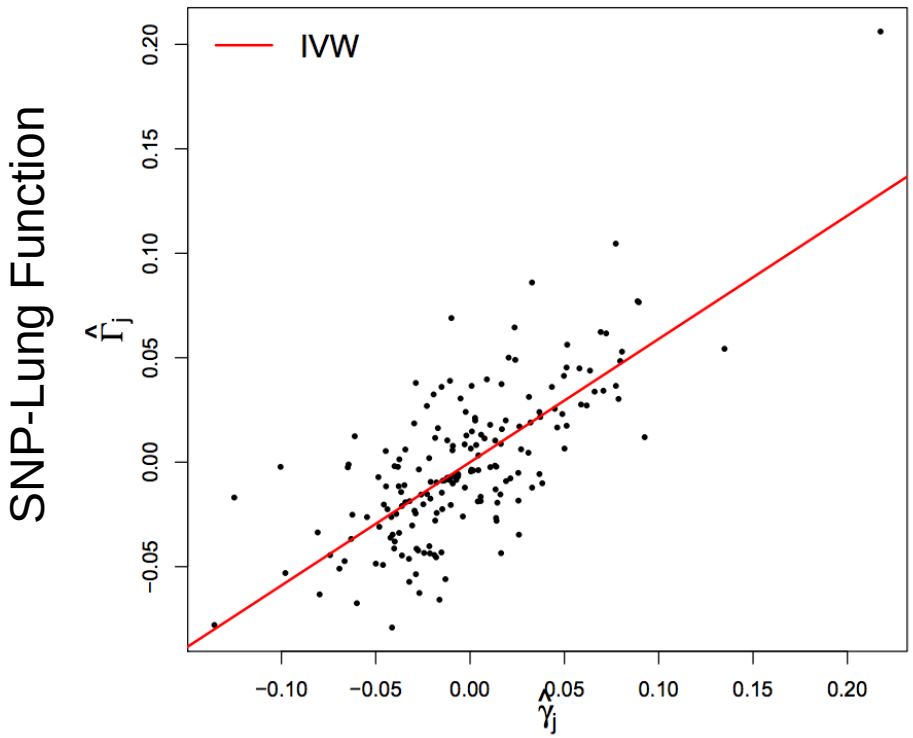
$$\hat{\Gamma}_j = \beta_{0E} + \beta_E \hat{\gamma}_j.$$

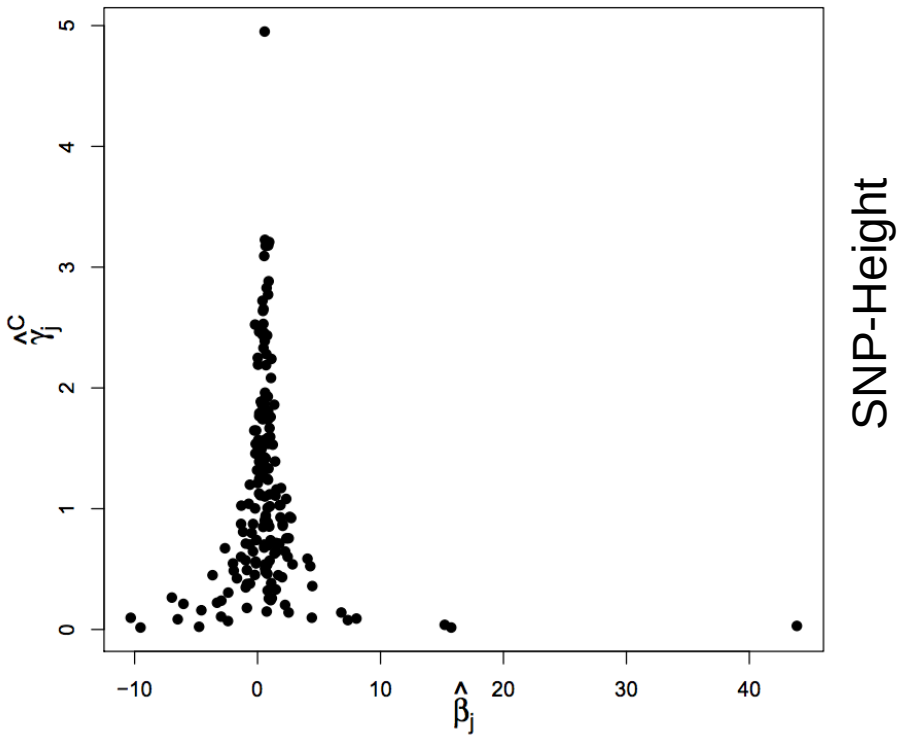
Intercept not constrained to zero



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 directional pleiotropy

Height and lung function



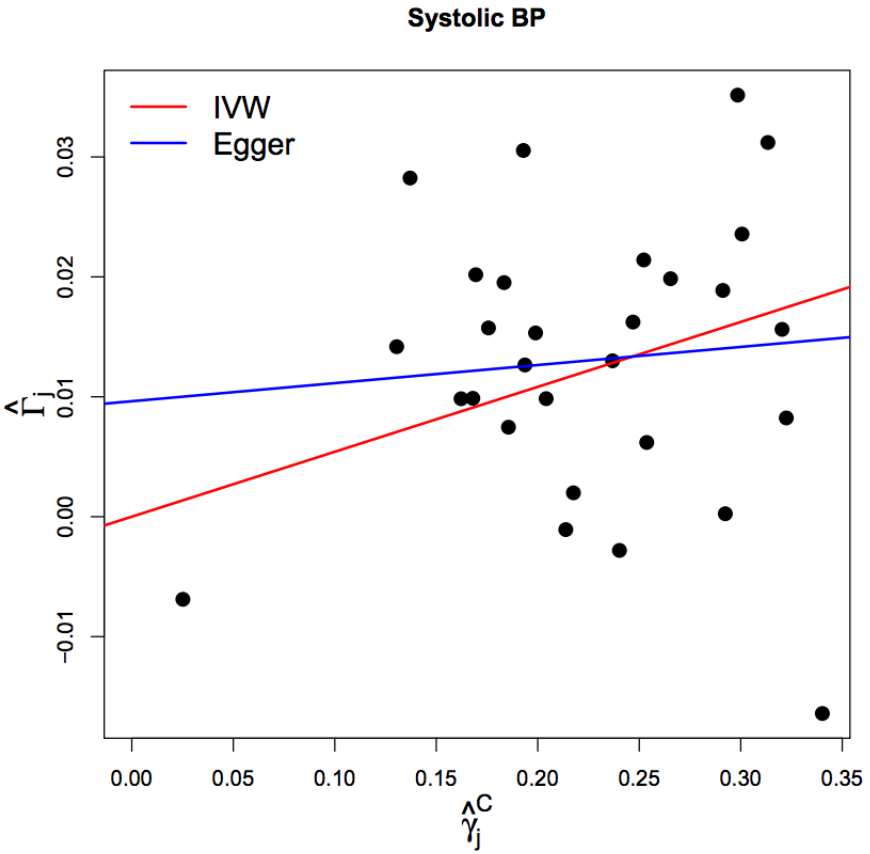


IVW = 0.59 (95% CI: 0.50, 0.67)

Egger = 0.58 (95% CI: 0.46, 0.75); intercept -0.001 p=0.5

BP and Coronary Disease

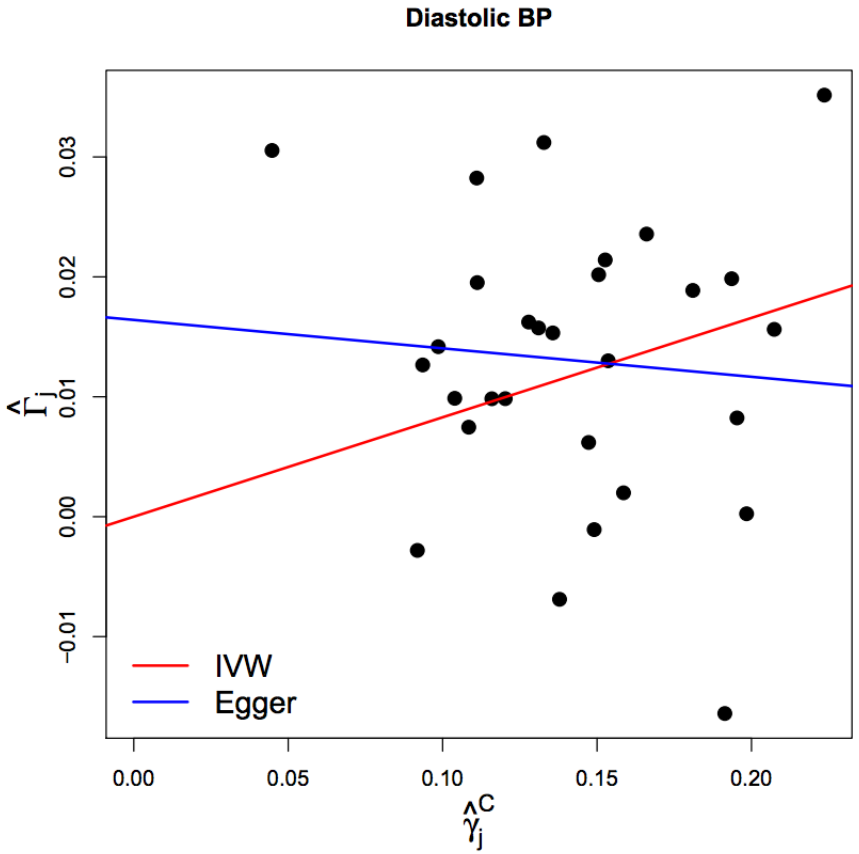
Scatter Plots



IVW= 0.054 logOR/mmHg p=4×10^{-6}

Egger =0.015 logOR/mmHg p=0.6

Egger test for intercept p=0.2



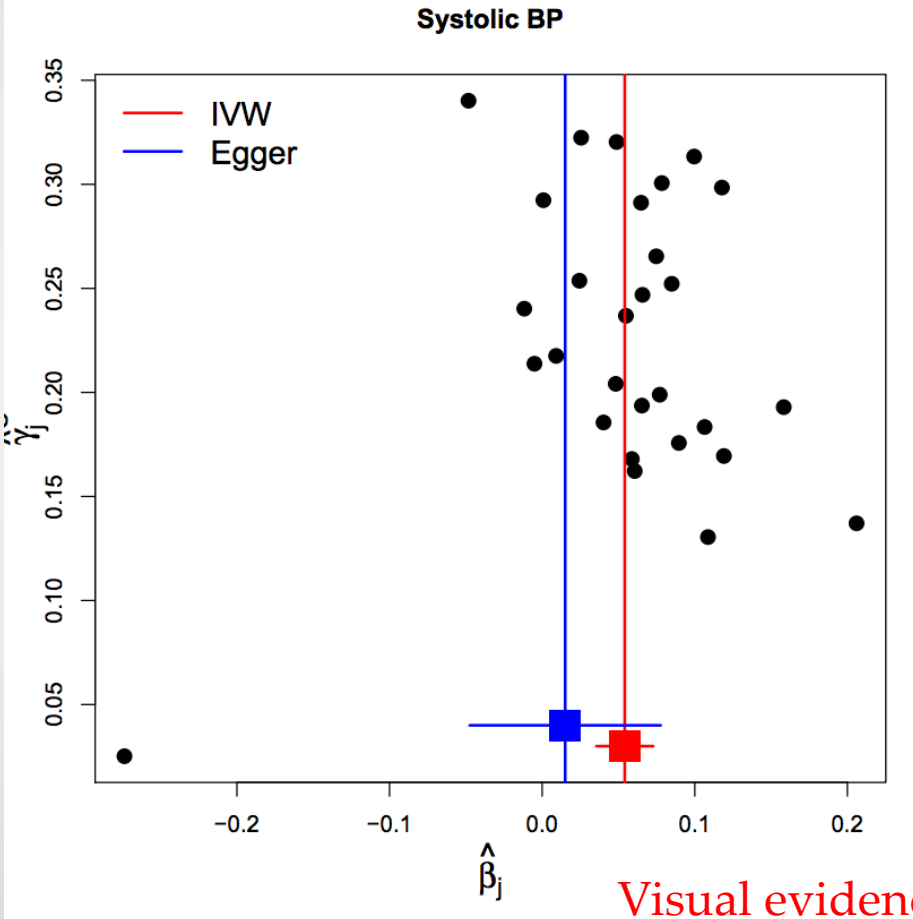
IVW= 0.083 logOR/mmHg p=1×10^{-5}

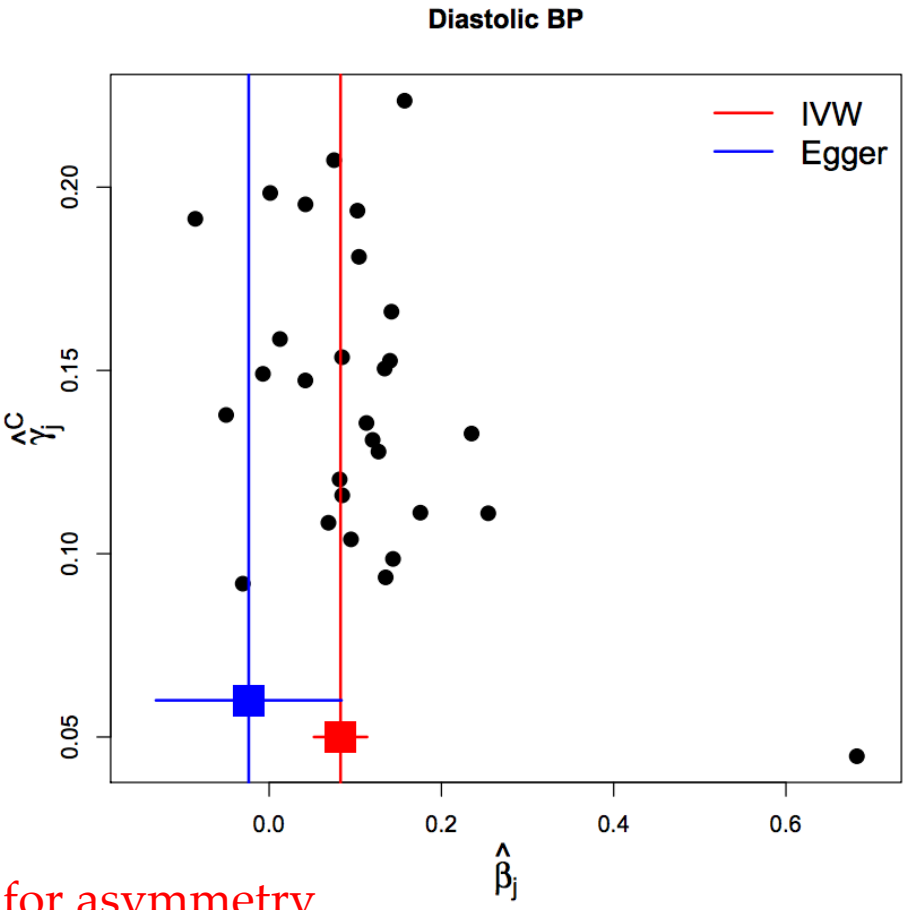
Egger =-0.024 logOR/mmHg p=0.7

Egger test for intercept p=0.054

BP and Coronary Disease

FUNNEL PLOTS





Visual evidence for asymmetry

IVW= 0.054 logOR/mmHg p=4×10^{-6}
Egger =0.015 logOR/mmHg p=0.6

IVW= 0.083 logOR/mmHg p=1×10^{-5}
Egger =-0.024 logOR/mmHg p=0.7

Median Estimator

Simple Median Method

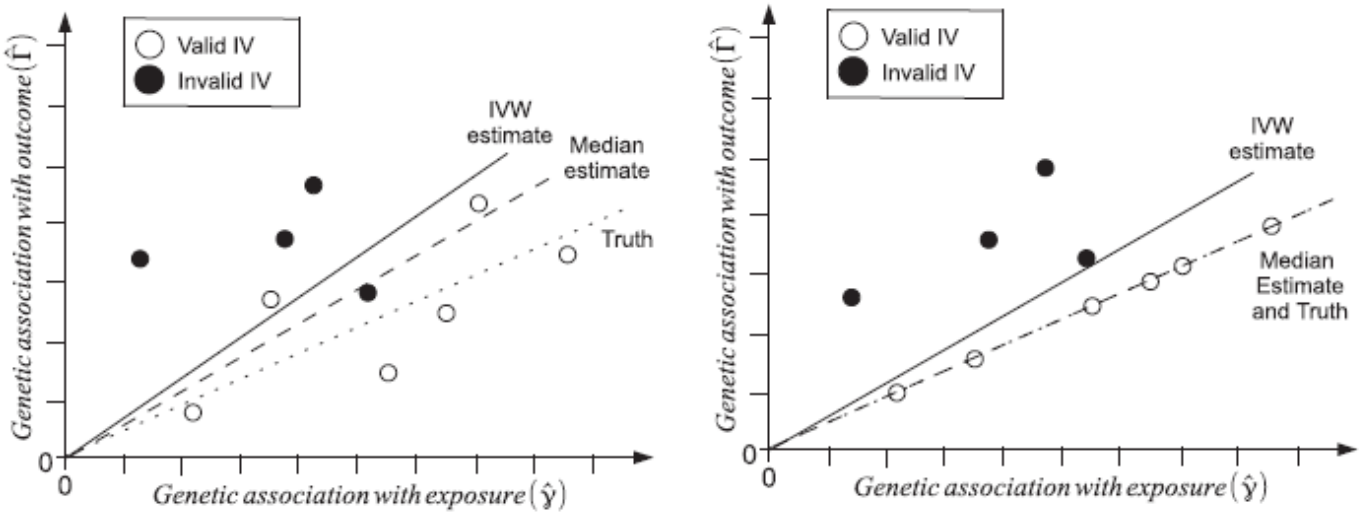


Figure 2. Fictional example of a Mendelian randomization analysis with 10 genetic variants—six valid instrumental variables (hollow circles) and four invalid instrumental variables (solid circles) for finite sample size (left) and infinite sample size (right) showing IVW (solid line) and simple median (dashed line) estimates compared with the true causal effect (dotted line). The ratio estimate for each genetic variant is the gradient of the line connecting the relevant datapoint for that variant to the origin; the simple median estimate is the median of these ratio estimates.

Order instrumental variables estimates and take the median

- Like all subsequent estimators it enjoys a 50% breakdown limit

Weighted Median Method

Table 1. Weights and percentiles of weighted median function

	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$	$\hat{\beta}_4$	$\hat{\beta}_5$	$\hat{\beta}_6$	$\hat{\beta}_7$	$\hat{\beta}_8$	$\hat{\beta}_9$	$\hat{\beta}_{10}$
Simple median										
Weight (w_j)	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$
Percentile (p_j)	5	15	25	35	45	55	65	75	85	95
Weighting 1										
Weight (w_j)	$\frac{1}{30}$	$\frac{2}{30}$	$\frac{3}{30}$	$\frac{4}{30}$	$\frac{5}{30}$	$\frac{5}{30}$	$\frac{4}{30}$	$\frac{3}{30}$	$\frac{2}{30}$	$\frac{1}{30}$
Percentile	1.67	6.67	15.00	26.67	41.67	58.33	73.33	85.00	93.33	98.33
Weighting 2										
Weight (w_j)	$\frac{2}{36}$	$\frac{3}{36}$	$\frac{10}{36}$	$\frac{8}{36}$	$\frac{5}{36}$	$\frac{3}{36}$	$\frac{2}{36}$	$\frac{1}{36}$	$\frac{1}{36}$	$\frac{1}{36}$
Percentile (p_j)	2.78	9.72	27.78	52.78	70.83	81.94	88.89	93.06	95.83	98.61

Weights and percentiles of the empirical distribution function assigned to the ordered ratio instrumental variable estimates ($\hat{\beta}_j$) for the hypothetical examples given in Figure 3.

Weighted Median Method

- Weights could be (normalized to add up to one) inverse variances of causal effect estimates

- SNPs that produce causal effect estimates that are outliers can be down-weighted => "Penalized Weighted Median method"

Mode Based Estimator

Simple Mode Based Estimator (MBE)

- Simple MBE: Group causal effect estimates by the similarity of their effect size. Choose the group that has the greatest number of SNPs.

- Relies on "ZEMPA" (**ZE**ro **M**odal **P**leiotropy **A**ssumption) to be a consistent estimator (i.e. the group that has the largest number of SNPs is also the group where there is no horizontal pleiotropy)

ZEMPA

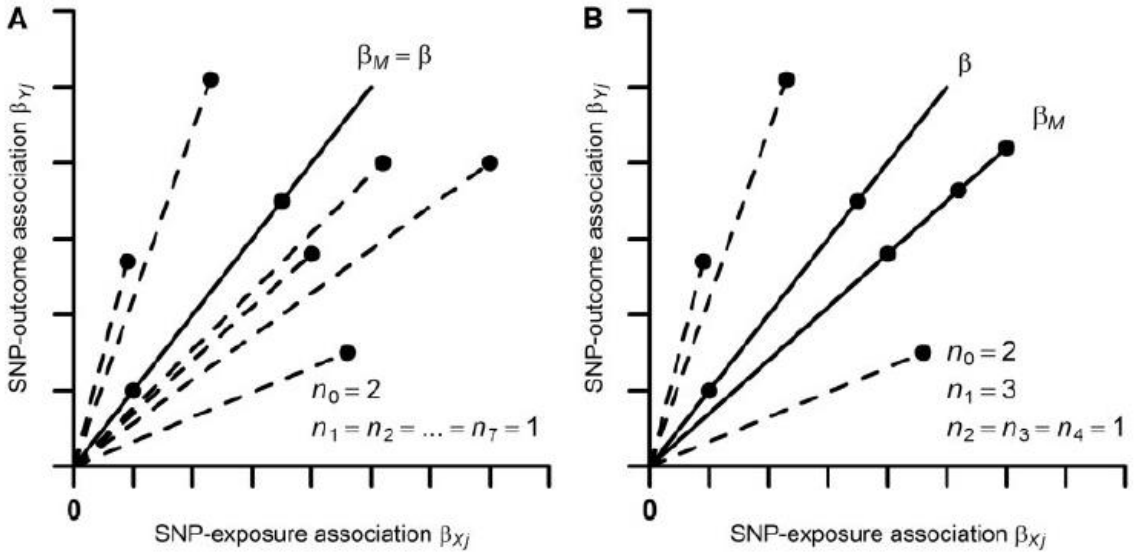
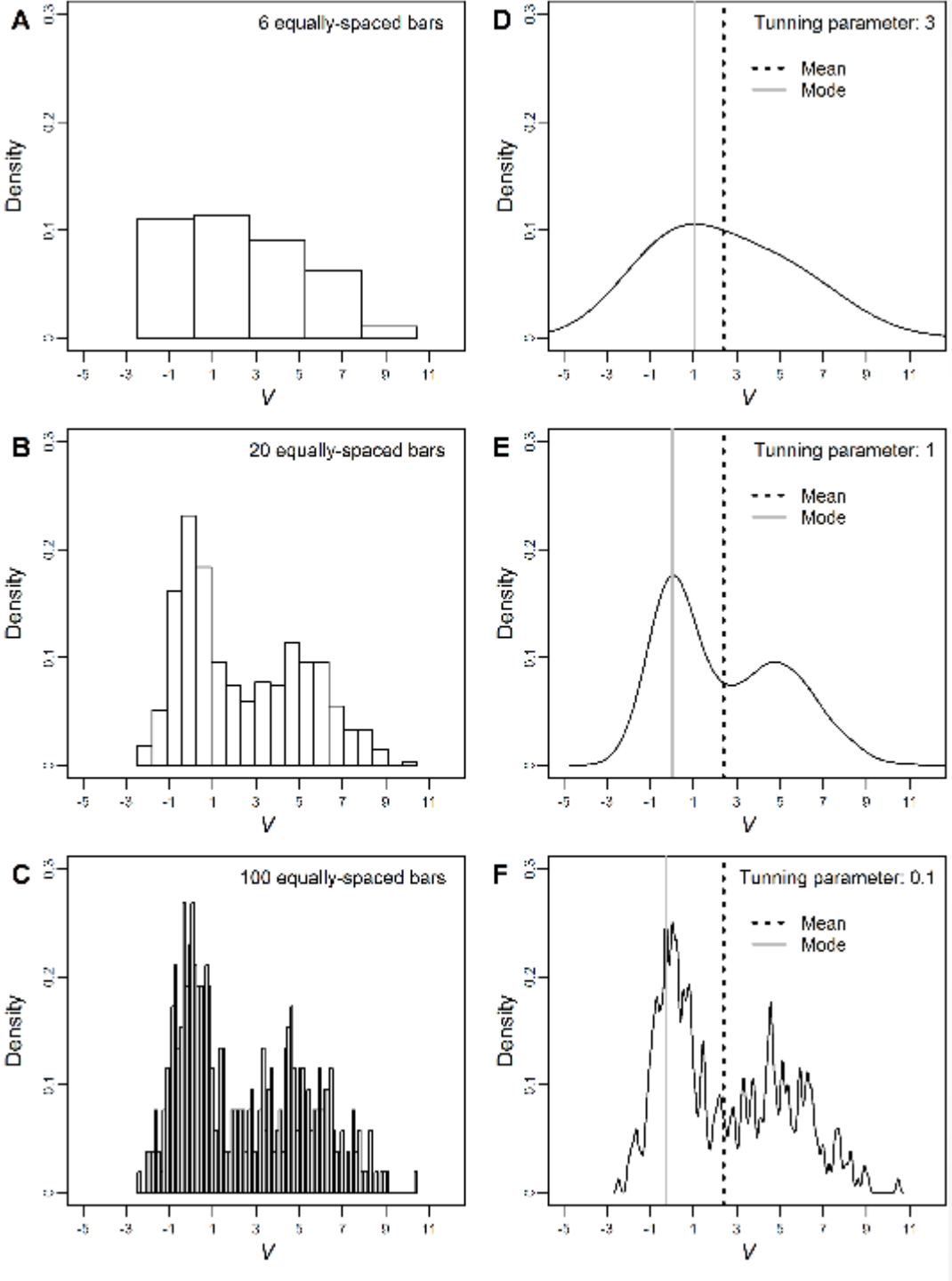


Figure 1. Illustration of the ZEro Modal Pleiotropy Assumption (ZEMPA) in the simple (i.e. unweighted) mode-based estimate (MBE). β_M is the simple MBE causal effect and β is the true causal effect; n_l denotes the number of variants with a given horizontal pleiotropic effect (n_0 denotes the number of valid instruments). Panel A: ZEMPA is satisfied. Panel B: ZEMPA is violated. SNP, single nucleotide polymorphism.

Weighted Mode Based Estimator

- It is possible to assign weights to variants (e.g. normalized inverse of variance of the causal effect)
- ZEMPA becomes that the sum of the weights associated with the valid instruments is the largest among all the different sub-groups of variants
- "Grouping" of variants by a procedure called "Kernel Density Estimation" (basically a way to estimate a probability density function)
- Take the value of the causal effect with the highest density


A
6 equally-spaced bars
B
20 equally-spaced bars
C
100 equally-spaced bars
D
Tunning parameter: 3
Mean
Mode
E
Tunning parameter: 1
Mean
Mode
F
Tunning parameter: 0.1
Mean
Mode
Density
V

International Journal of Epidemiology, 2017, 1985–1998
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Original article

Methods

Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption

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Abstract

Background: Mendelian randomization (MR) is being increasingly used to strengthen causal inference in observational studies. Availability of summary data of genetic associations for a variety of phenotypes from large genome-wide association studies (GWAS) allows straightforward application of MR using summary data methods, typically in a two-sample design. In addition to the conventional inverse variance weighting (IVW) method, recently developed summary data MR methods, such as the MR-Egger and weighted median approaches, allow a relaxation of the instrumental variable assumptions.

Methods: Here, a new method - the mode-based estimate (MBE) - is proposed to obtain a single causal effect estimate from multiple genetic instruments. The MBE is consistent when the largest number of similar (identical in infinite samples) individual-instrument causal effect estimates comes from valid instruments, even if the majority of instruments are invalid. We evaluate the performance of the method in simulations designed to mimic the two-sample summary data setting, and demonstrate its use by investigating the causal effect of plasma lipid fractions and urate levels on coronary heart disease risk.

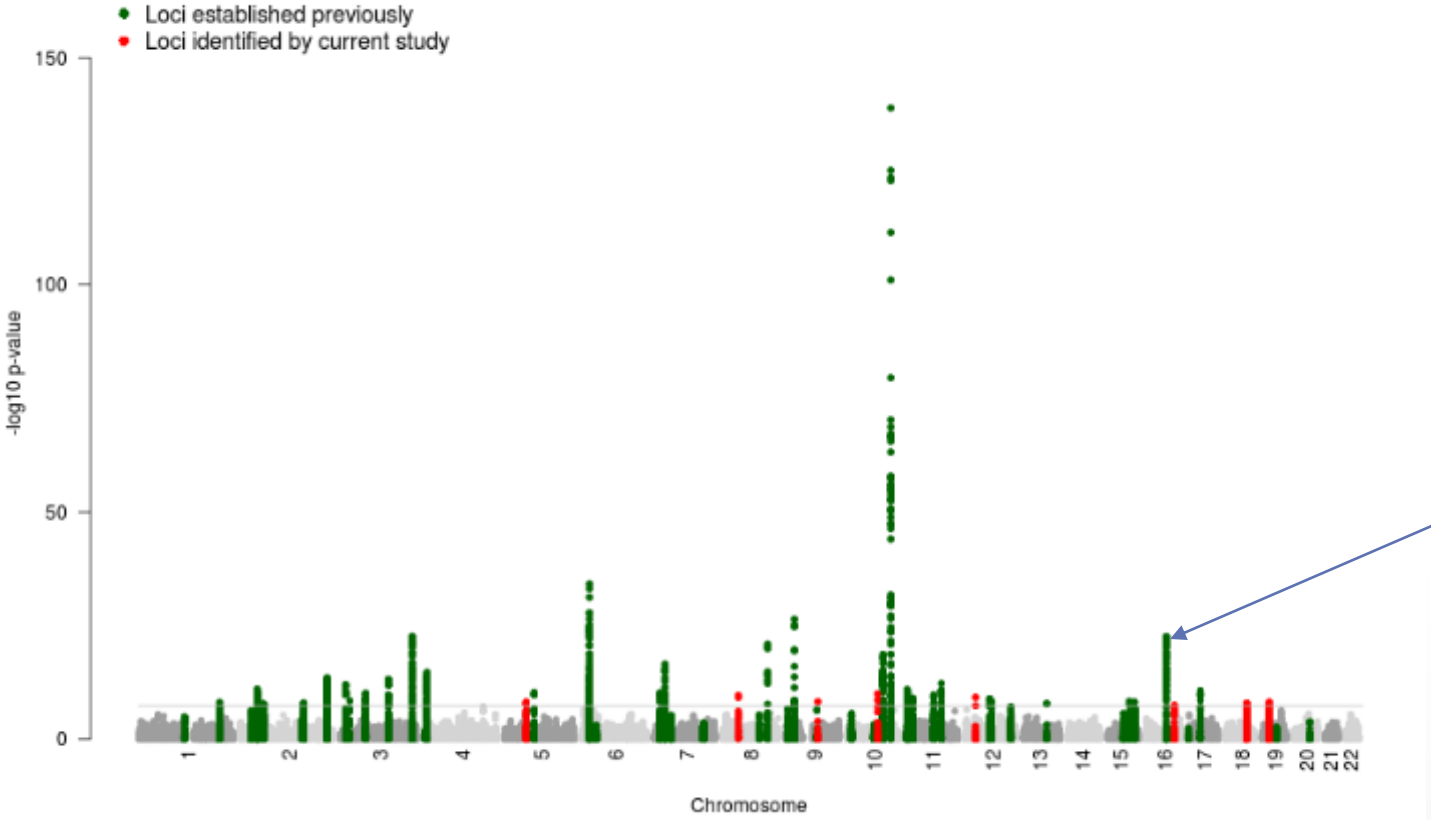
Results: The MBE presented less bias and lower type-I error rates than other methods under the null in many situations. Its power to detect a causal effect was smaller compared with the IVW and weighted median methods, but was larger than that of MR-Egger regression, with sample size requirements typically smaller than those available from GWAS consortia.

Conclusions: The MBE relaxes the instrumental variable assumptions, and should be used in combination with other approaches in sensitivity analyses.

Key words: Causality, instrumental variables, genetic variation, Mendelian randomization, genetic pleiotropy

Reverse causal instruments?

Problem: MR of type 2 diabetes on BMI



GWAS of T2D reveals *FTO* variant - Famously associated with BMI

Can we avoid including reverse-causal SNPs as instruments?

- If a SNP is correlated with an exposure "variable B" and variable B causes "variable C", then the correlation between the SNP and variable B should be larger than the correlation between the SNP and variable C
- A "Steiger test" can be performed that examines whether the SNP-outcome correlation is greater than the SNP-exposure correlation
- SNPs that fail this test may not be primarily associated with the exposure, and can be filtered before analysis

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RESEARCH ARTICLE

Orienting the causal relationship between imprecisely measured traits using GWAS summary data

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Data Availability Statement: All scripts and data used to perform these analyses are available without restriction at https://github.com/explodecomputer/causal-directions. The summary data used in the main analysis was obtained from a previously published study (Shakhbazov et al 2016) and can be downloaded from https://static-content.springer.com/esm/art%3A10.1186%2Fs12864-016-2498-4/MediaObjects/12864_2016_2498_MOESM8_ESM.csv and https://static-content.springer.com/esm/art%3A10.1186%2Fs12864-016-2498-4/MediaObjects/12864_2016_2498_MOESM16_ESM.csv.

Abstract

Inference about the causal structure that induces correlations between two traits can be achieved by combining genetic associations with a mediation-based approach, as is done in the causal inference test (CIT). However, we show that measurement error in the phenotypes can lead to the CIT inferring the wrong causal direction, and that increasing sample sizes has the adverse effect of increasing confidence in the wrong answer. This problem is likely to be general to other mediation-based approaches. Here we introduce an extension to Mendelian randomisation, a method that uses genetic associations in an instrumentation framework, that enables inference of the causal direction between traits, with some advantages. First, it can be performed using only summary level data from genome-wide association studies; second, it is less susceptible to bias in the presence of measurement error or unmeasured confounding. We apply the method to infer the causal direction between DNA methylation and gene expression levels. Our results demonstrate that, in general, DNA methylation is more likely to be the causal factor, but this result is highly susceptible to bias induced by systematic differences in measurement error between the platforms, and by horizontal pleiotropy. We emphasise that, where possible, implementing MR and appropriate sensitivity analyses alongside other approaches such as CIT is important to triangulate reliable conclusions about causality.

Author summary

Understanding the causal relationships between pairs of traits is crucial for unravelling the causes of disease. To this end, results from genome-wide association studies are valuable because if a trait is known to be influenced by a genetic variant then this knowledge can be used to test the trait's causal influences on other traits and diseases. Here we discuss scenarios where the nature of the genetic association with the causal trait can lead existing causal inference methods to give the wrong direction of causality. We introduce a new method that can be applied to summary level data and is potentially less susceptible to problems such as measurement error, and apply it to evaluate the causal relationships between DNA methylation levels and gene expression. While our results show that DNA

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

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