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

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

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

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

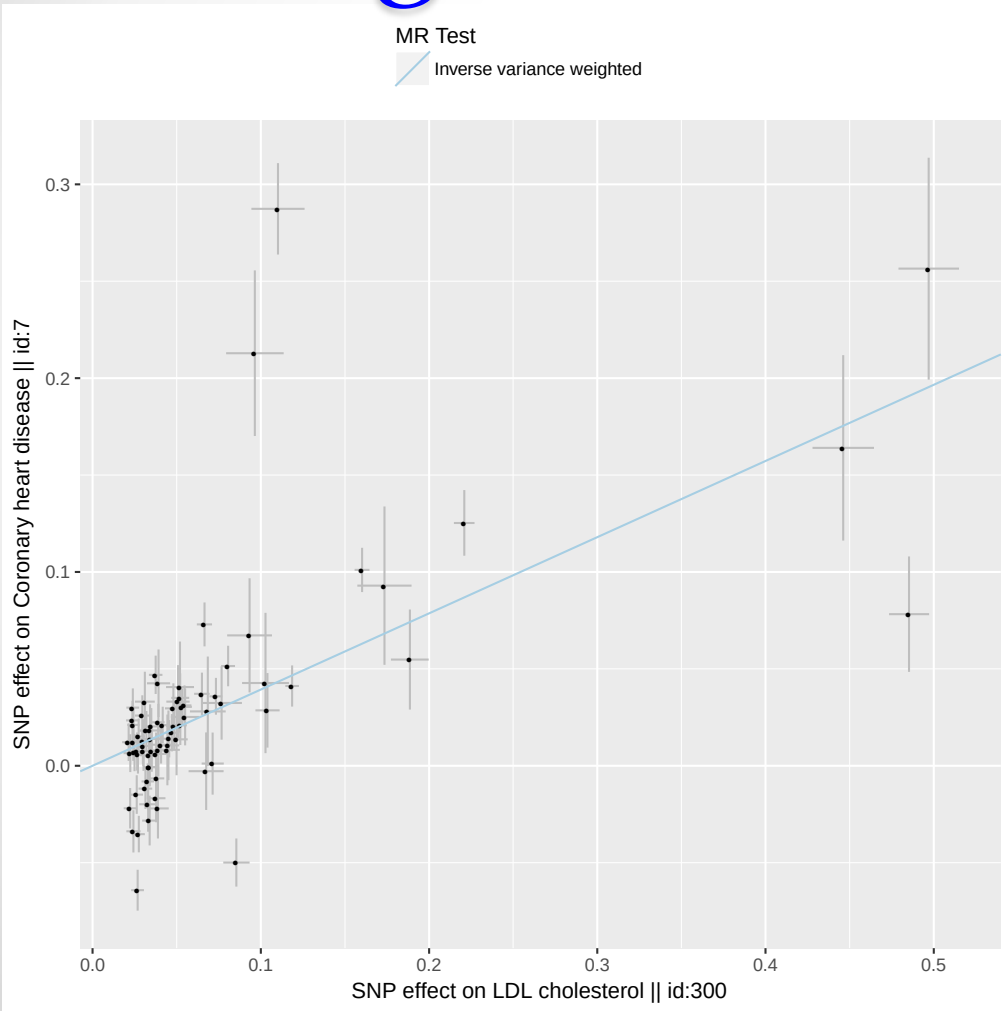
For N studies, each study *i* contributes more to the meta analysis if its standard error is lower

Calculate p-value

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

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

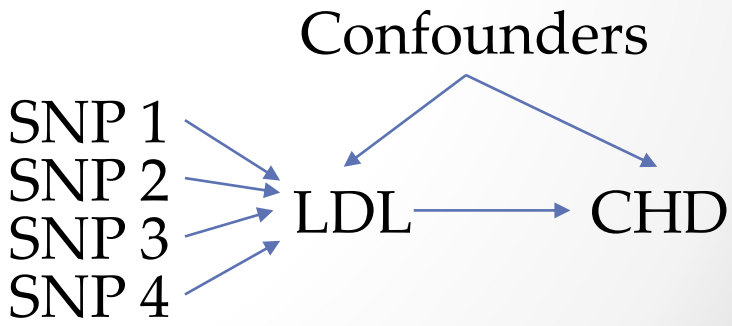
Fixed Effects IVW-MR and Weighted Linear regression



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

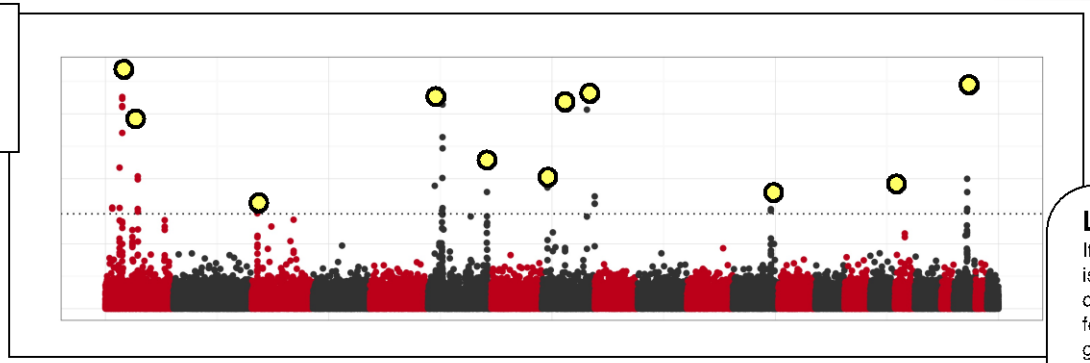
The weights are 1/SE_SNP_outcome

The slope is the estimate of the causal effect

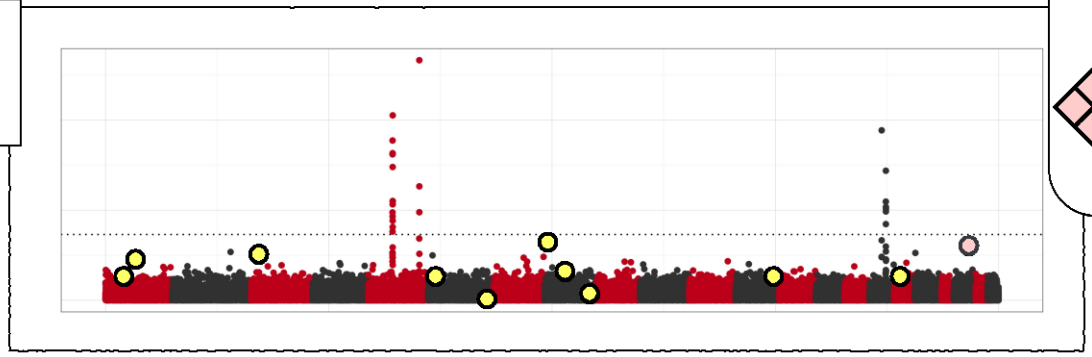


Performing MR With Summary Statistics

Obtain instruments from exposure GWAS

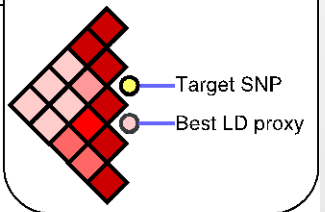


Extract SNP effects from outcome GWAS

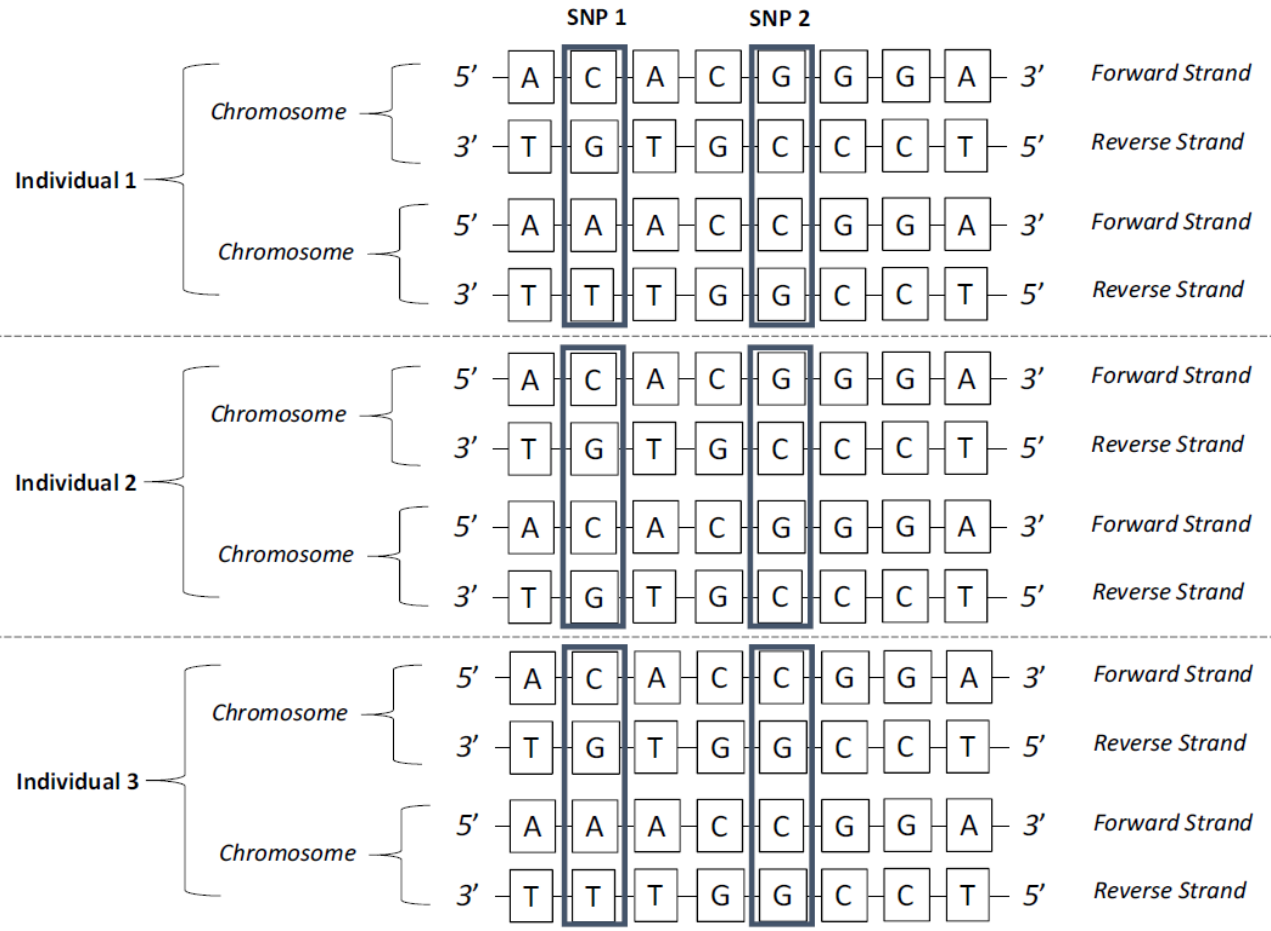


LD Proxies

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



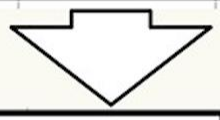
The Issue of Strand



Evans et al (2021) *Behav Genet*

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**

Strand issue exercise

SNP	Study 1 alleles	Study 1 allele freq	Study 2 alleles	Study 2 allele freq	Verdict
rs1	A/G	0.2	A/G	0.2	
rs2	G/T	0.3	T/G	0.72	
rs3	G/C	0.65	G/C	0.62	
rs4	A/T	0.49	A/T	0.50	
rs5	A/T	0.12	A/T	0.89	
rs6	A/G	0.4	A/T	0.4	

MR methods for handling horizontal pleiotropy

...

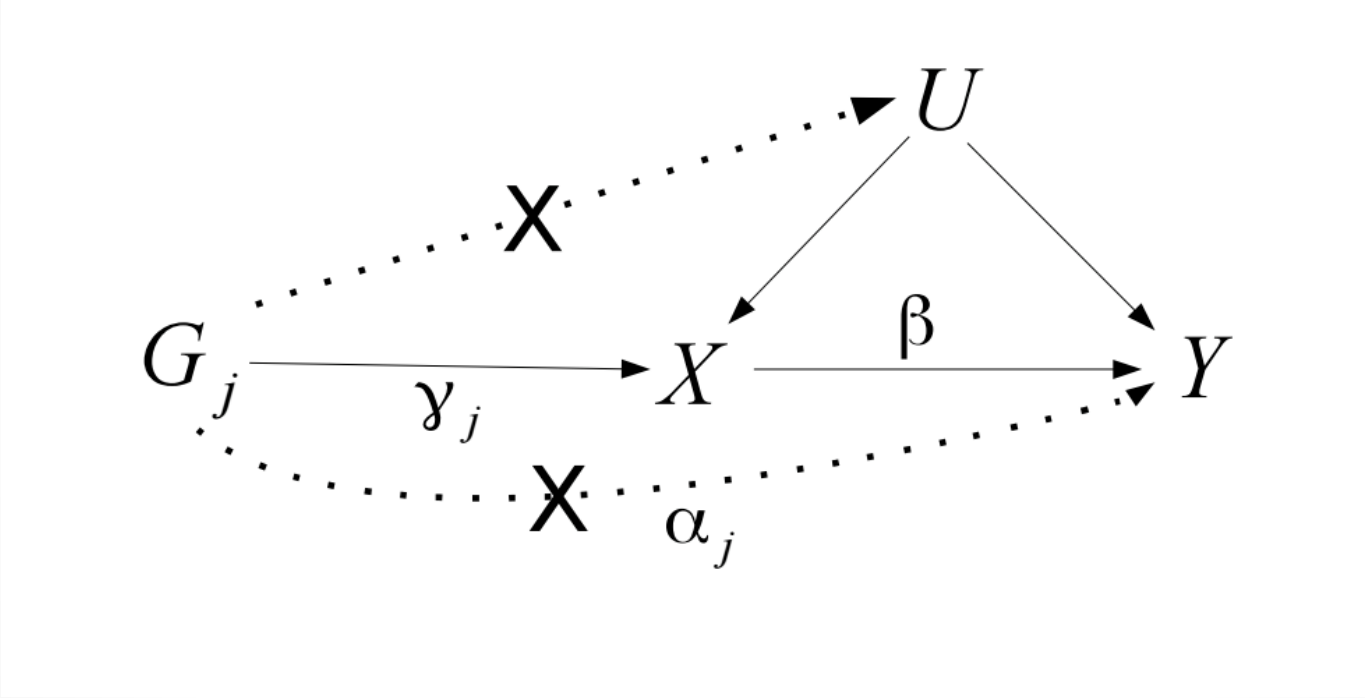
Many methods now exist

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



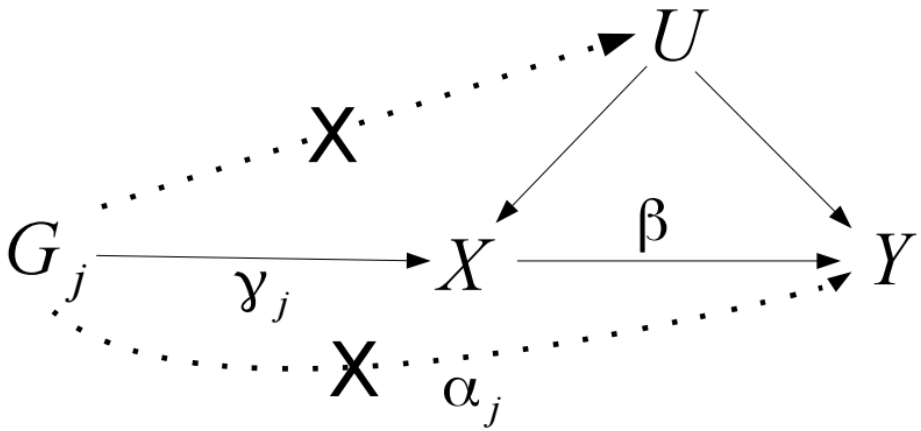
Wald = Beta-GY / Beta-GX

Causal estimate using Wald method:

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

Two Sample MR:

Multiple Variants



Causal estimate using IVW from summarised data:

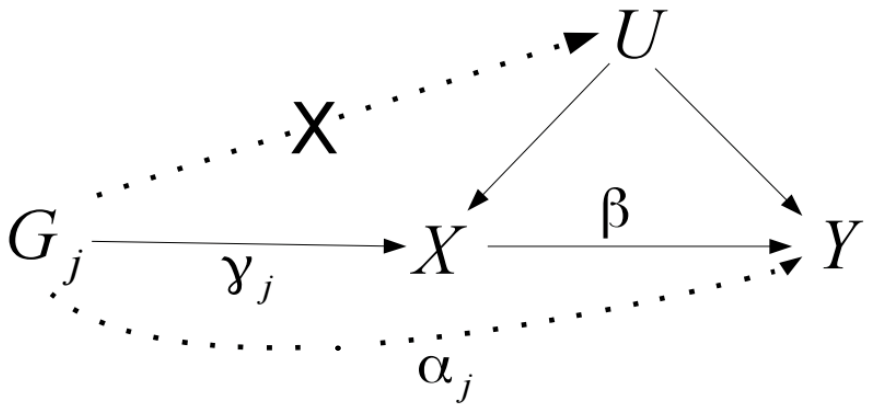
$$\frac{\sum_{j=1}^{J} \hat{\gamma}_j^2 \sigma_{Yj}^{-2} \hat{\beta}_j}{\sum_{j=1}^{J} \hat{\gamma}_j^2 \sigma_{Yj}^{-2}} = \beta.$$

(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 :

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

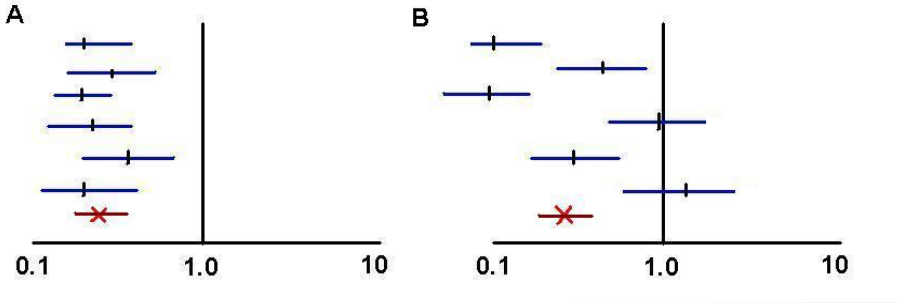
Heterogeneity

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

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



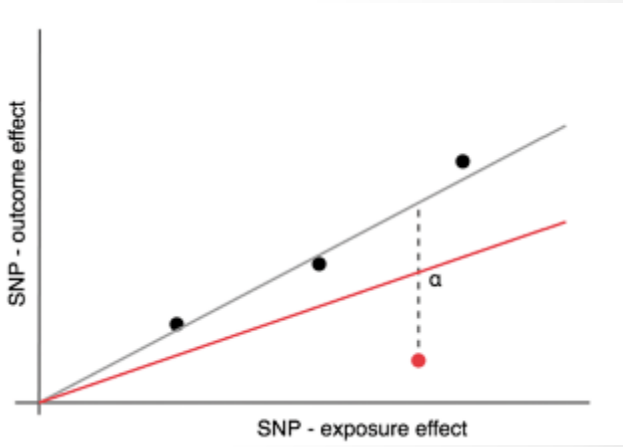
n=6 instruments
Expect Q = 5 if there is no heterogeneity
Q is chi-square distributed with n-1 degrees of freedom

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

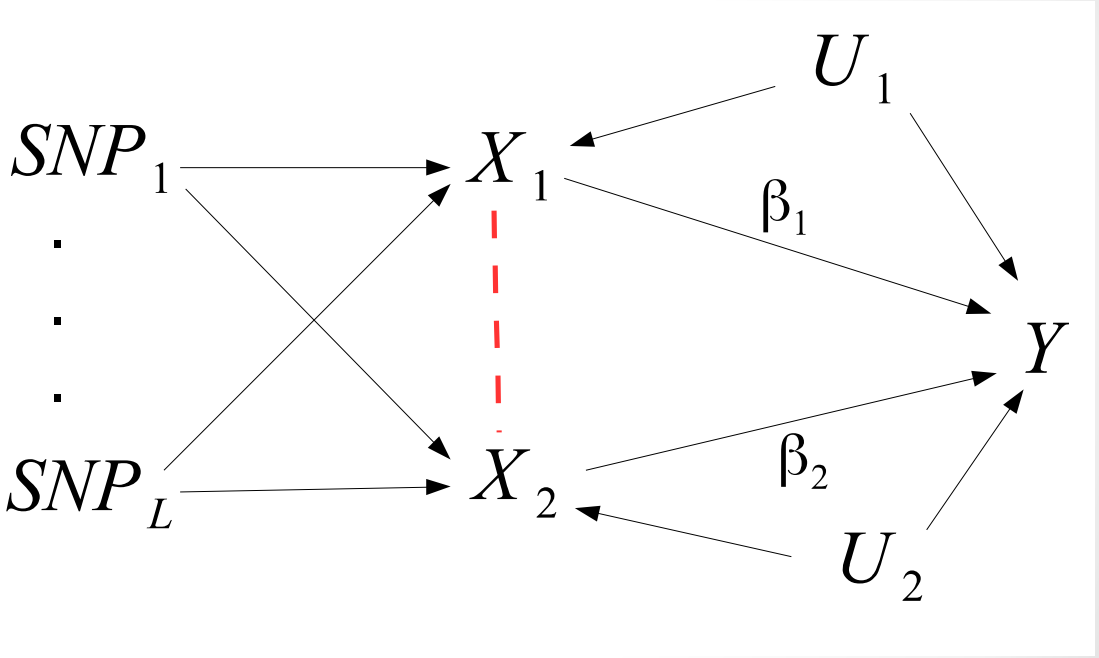
However – beware of:

- 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…

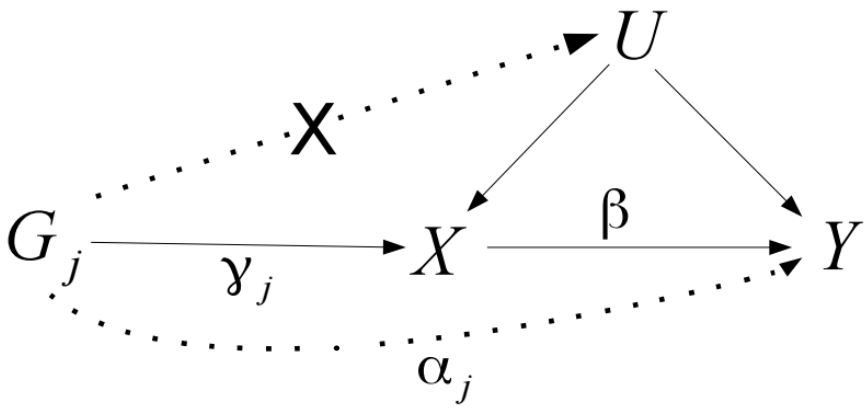
MR Egger Regression

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)

InSIDE Assumption

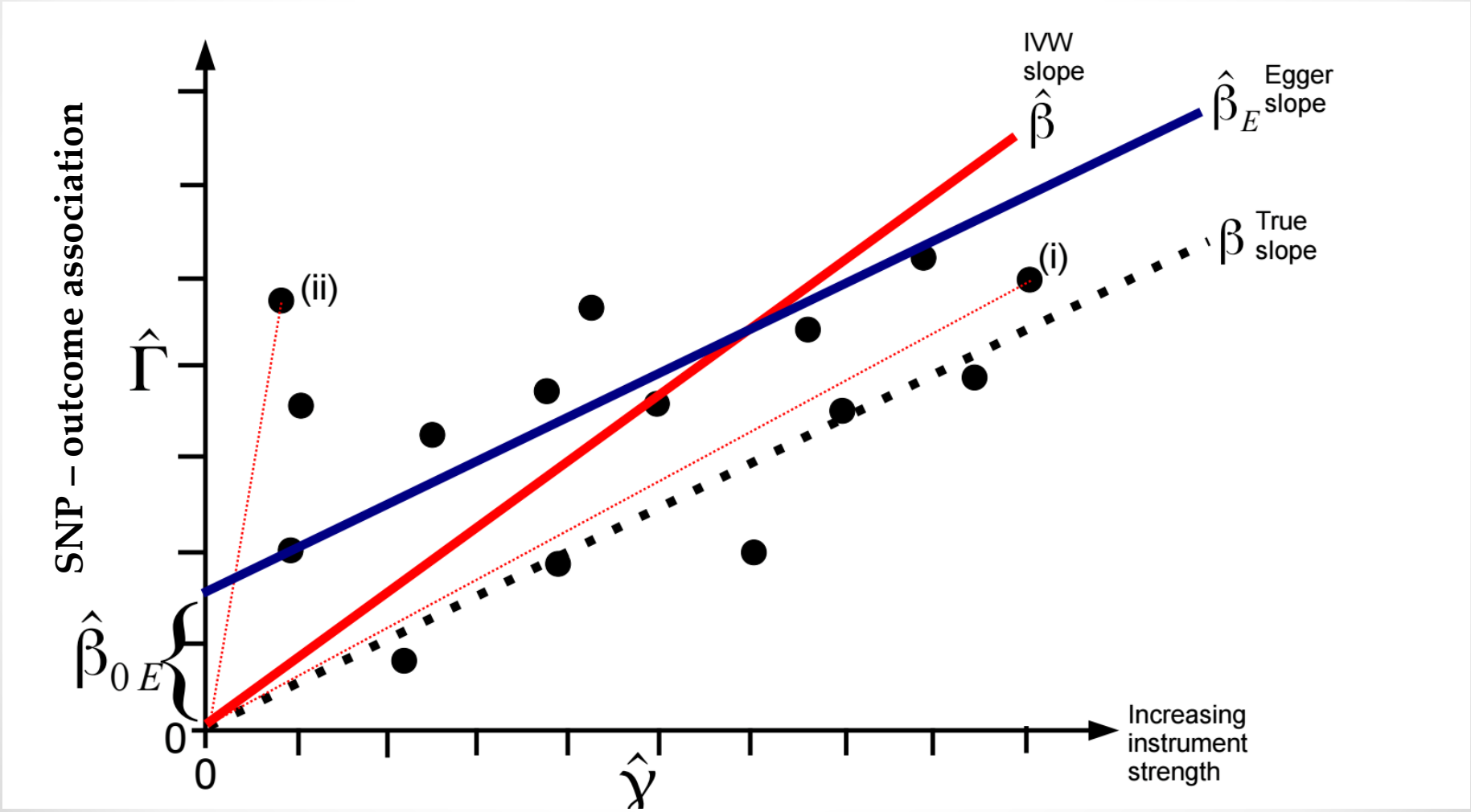
Relaxing MR's assumptions



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

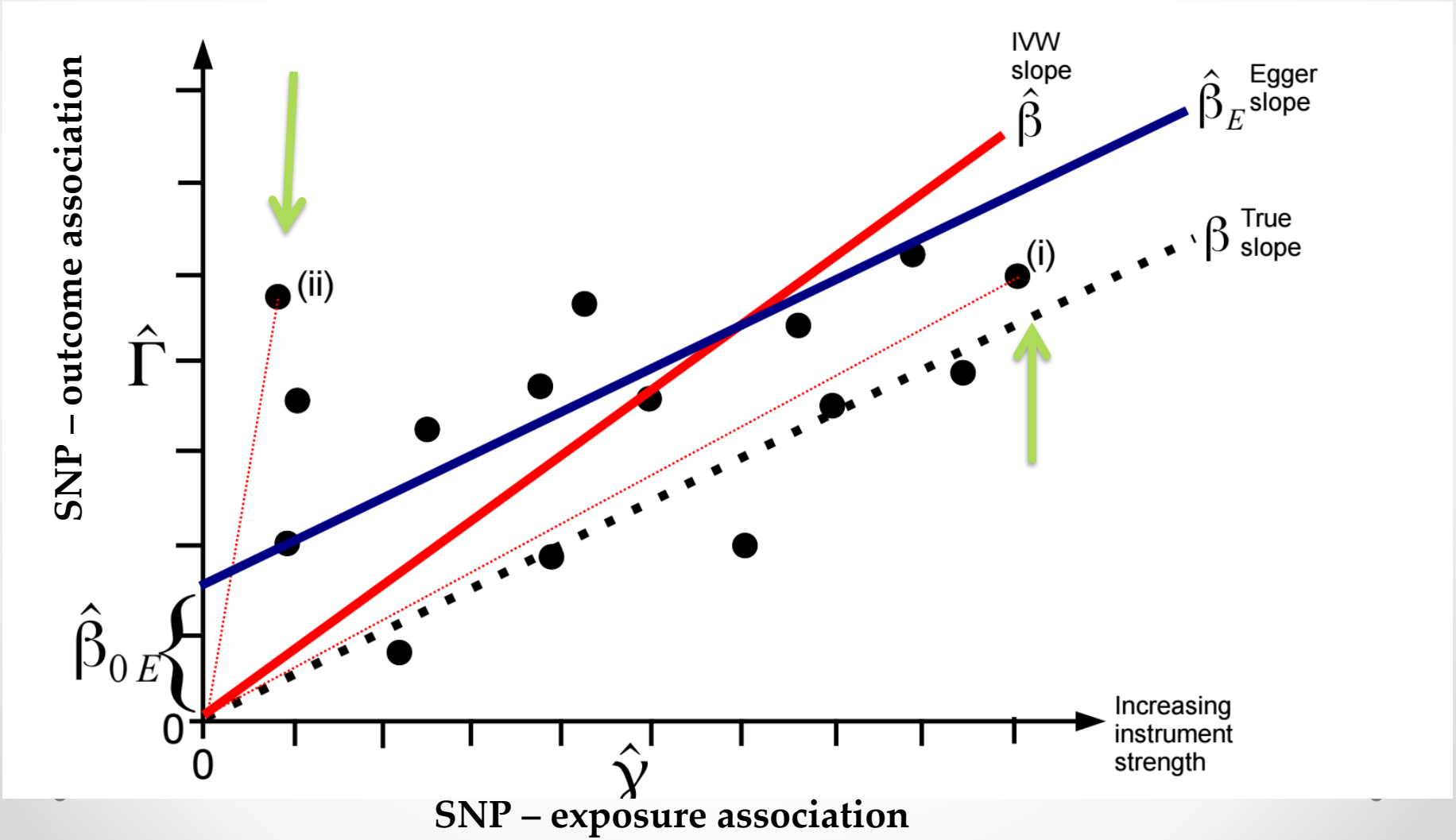
Example:

ALL INVALID INSTRUMENTS
INSIDE ASSUMPTION SATISFIED



InSIDE: $\hat{\alpha}_j$ is independent of its denominator, $\hat{\gamma}_j$.

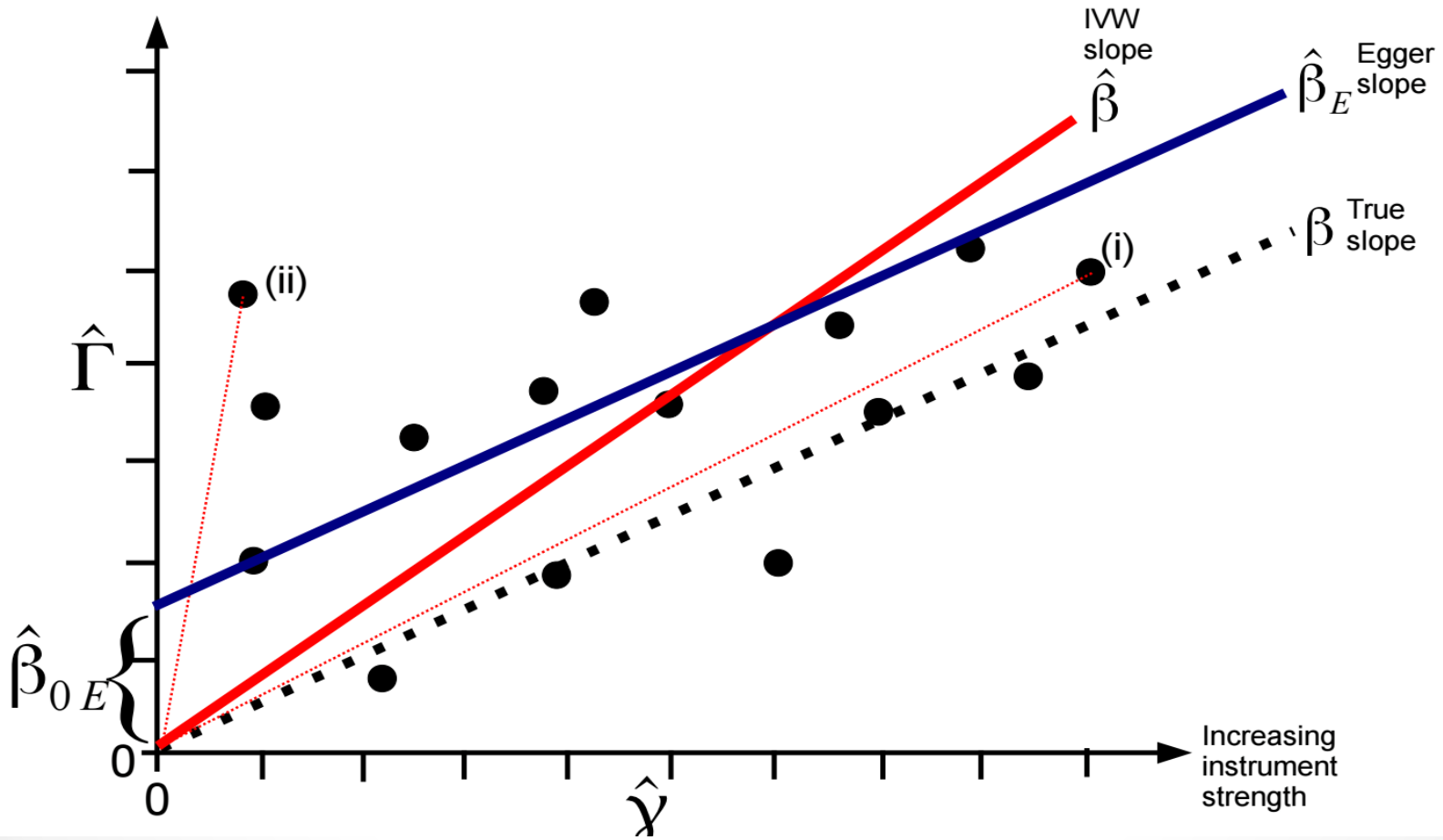
Bias of ratio estimator $\hat{\beta}_j = \frac{\hat{\Gamma}_j}{\hat{\gamma}_j}$ is inversely proportional to γ_j.



Egger regression:

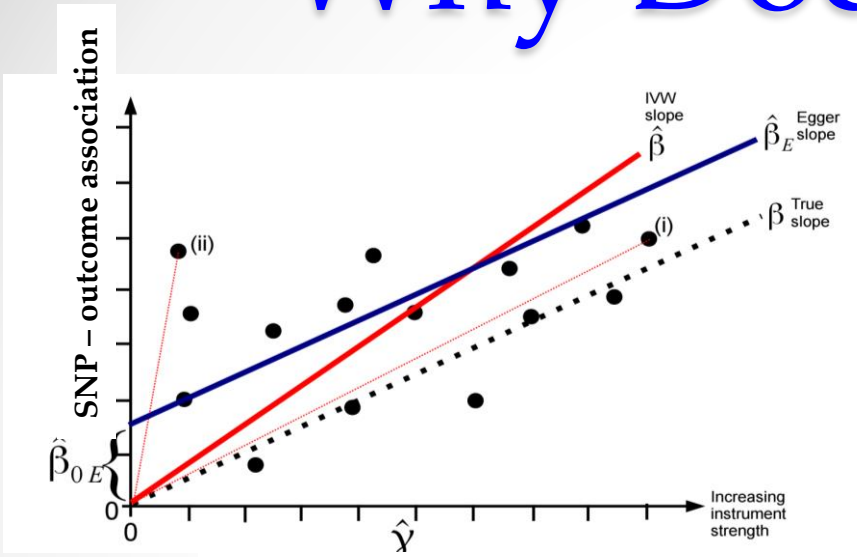
$$\hat{\Gamma}_j = \beta_{0E} + \beta_E \hat{\gamma}_j.$$

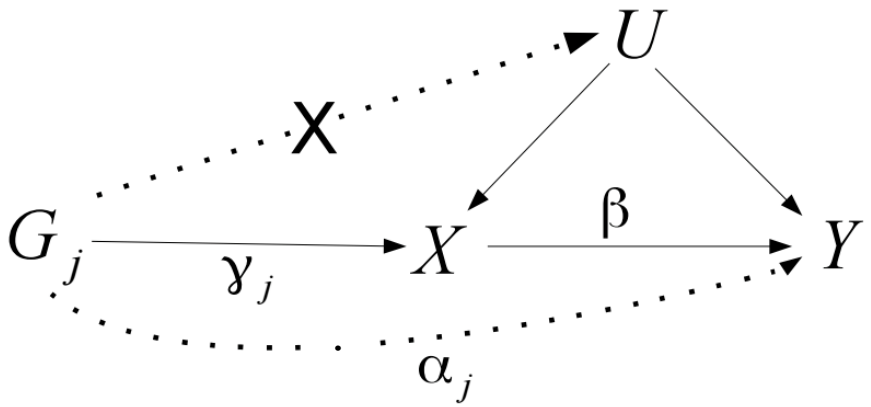
Intercept not constrained to zero



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

Why Does It Work?





$$\beta_{Egger} = \frac{cov(\hat{\beta}_{SNP-OUTCOME}, \hat{\beta}_{SNP-EXPOSURE})}{var(\hat{\beta}_{SNP-EXPOSURE})}$$

$$= \frac{cov(\hat{\beta}\hat{\gamma}_i + \hat{\alpha}_i, \hat{\gamma}_i)}{var(\hat{\gamma}_i)}$$

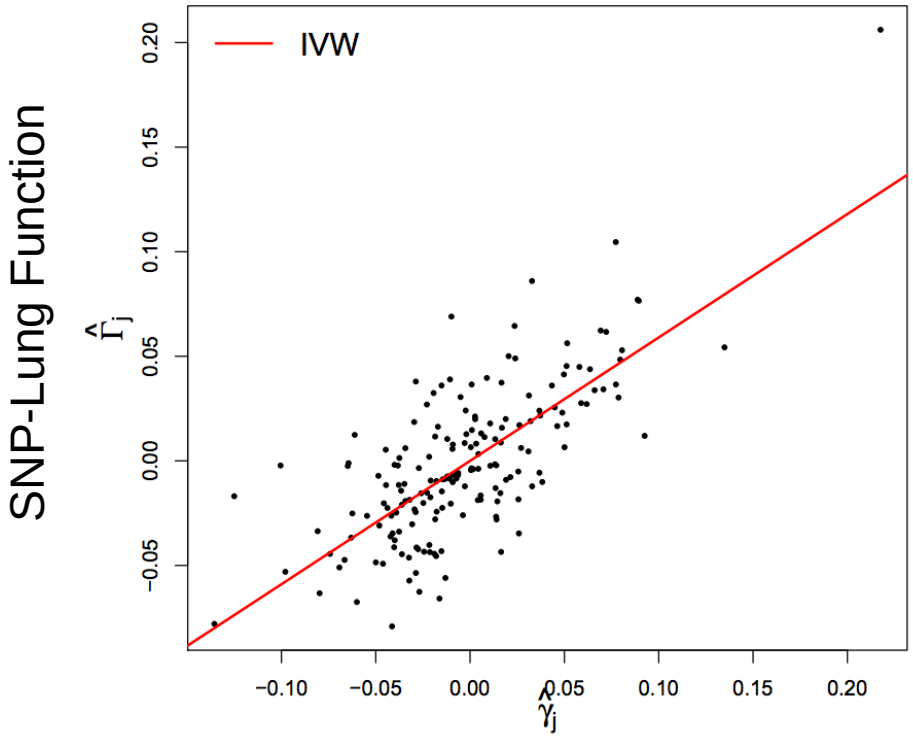
$$= \frac{\hat{\beta}cov(\hat{\gamma}_i, \hat{\gamma}_i) + cov(\hat{\alpha}_i, \hat{\gamma}_i)}{var(\hat{\gamma}_i)}$$

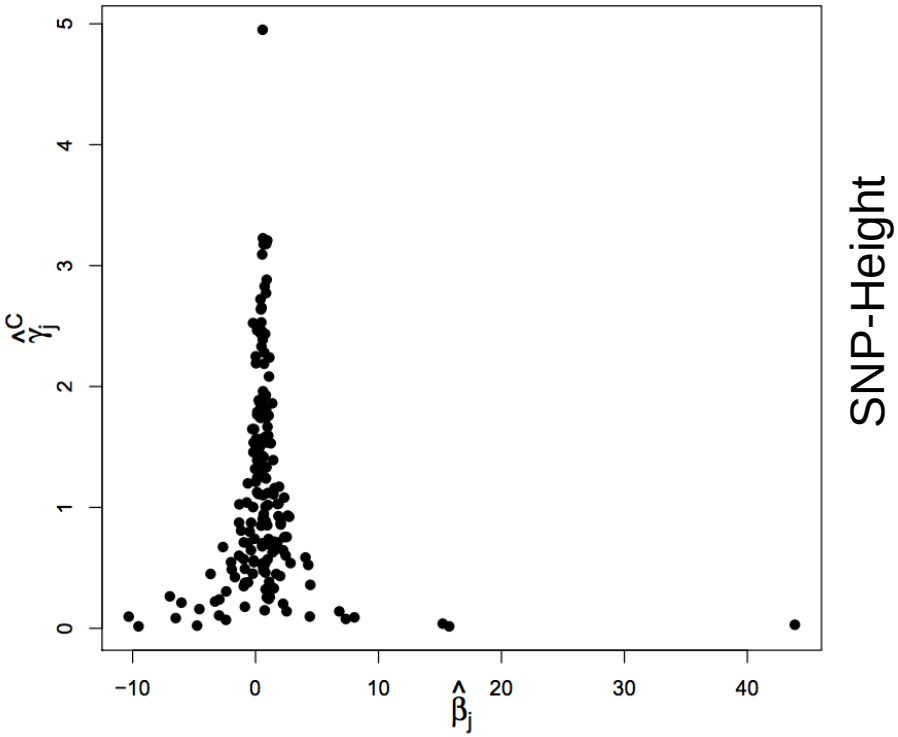
$$= \hat{\beta} + \frac{cov(\hat{\alpha}_i, \hat{\gamma}_i)}{var(\hat{\gamma}_i)}$$

As N $\rightarrow \infty$, $\text{cov}(\hat{\alpha}_i, \hat{\gamma}_i) \rightarrow \text{cov}(\alpha_i, \gamma_i)$

As #markers $\rightarrow \infty$, $\text{cov}(\alpha_i, \gamma_i) \rightarrow 0$

Height and lung function



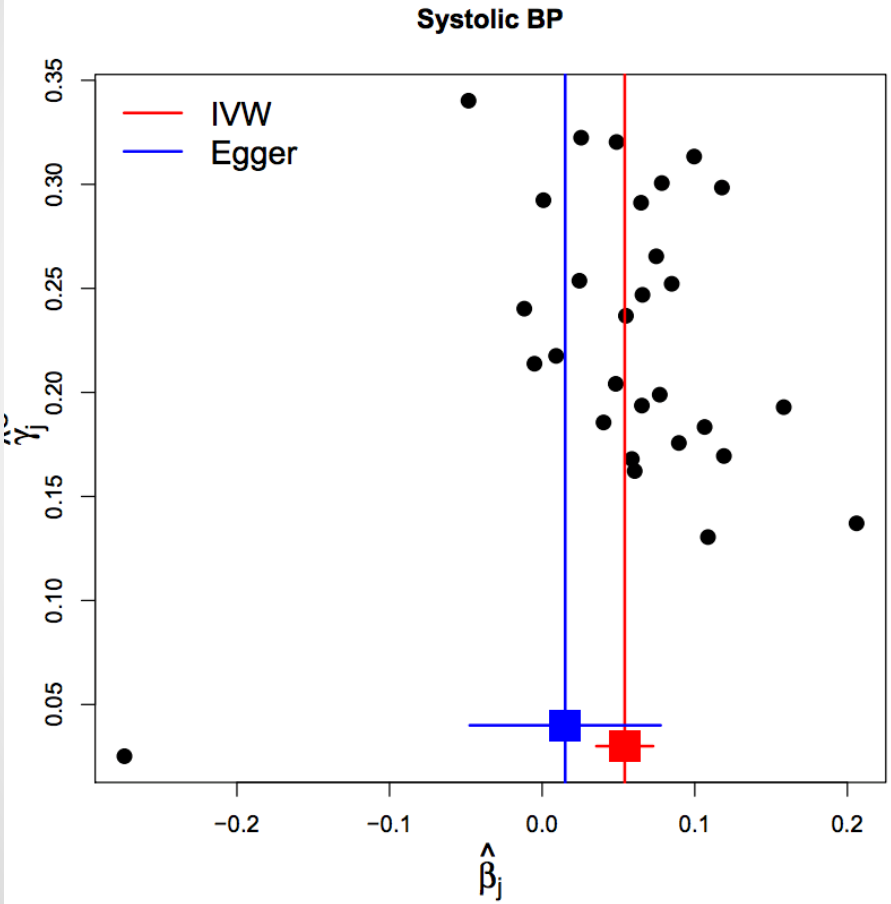


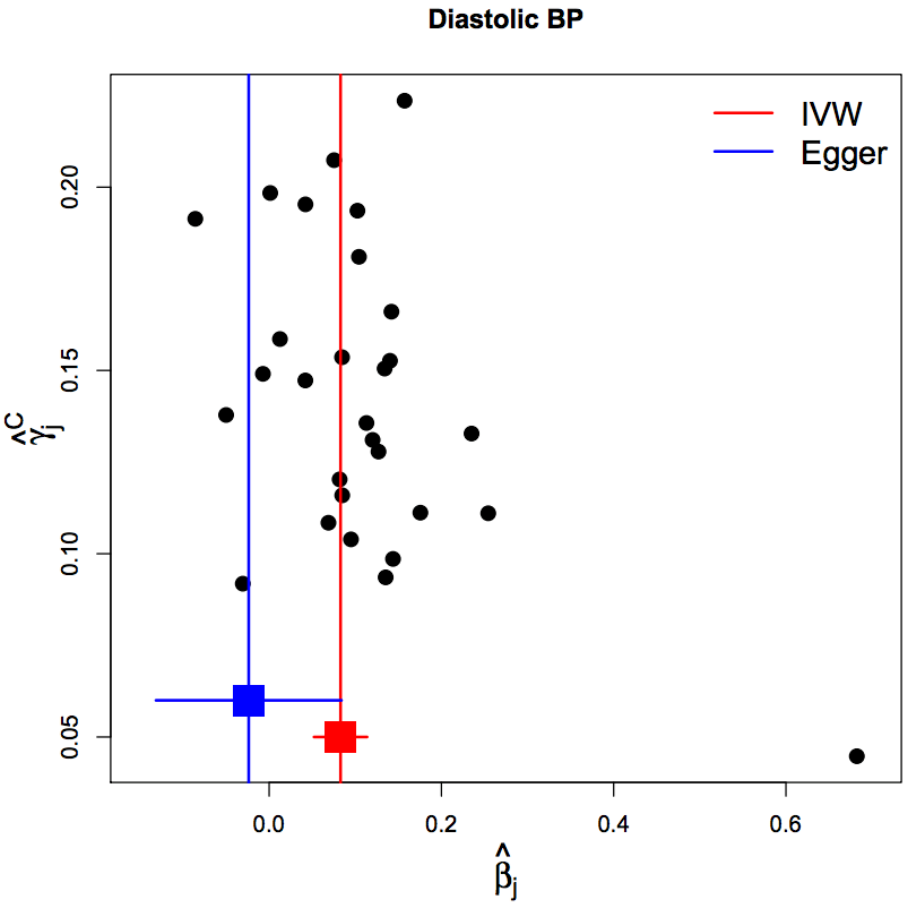
IVW = 0.59 (95% CI: 0.50, 0.67)

Egger = 0.58 (95% CI: 0.50, 0.67); intercept -0.001 p=0.5

BP and Coronary Disease

FUNNEL PLOTS

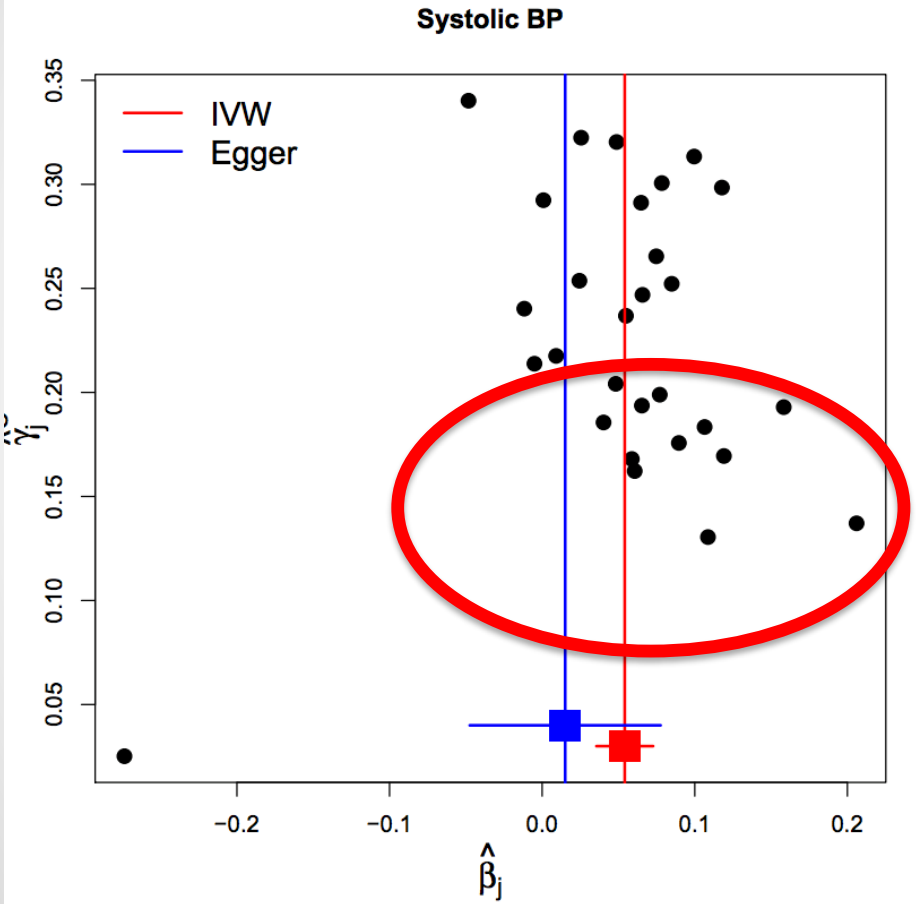


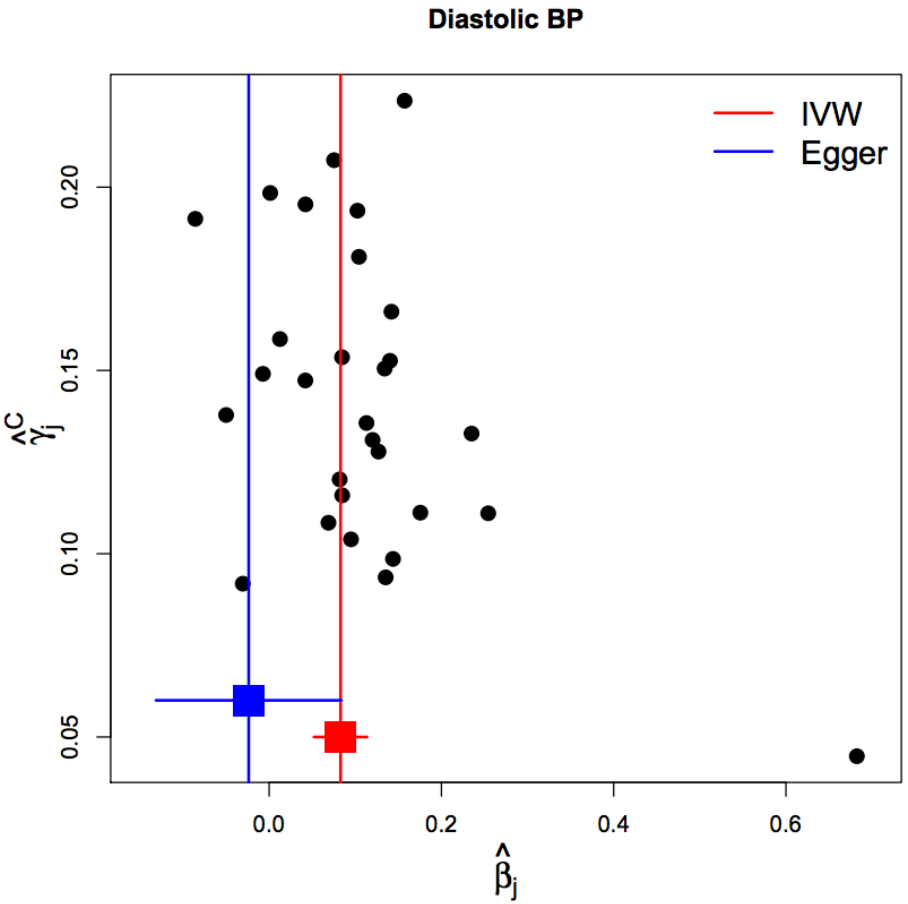


Visual evidence for asymmetry

BP and Coronary Disease

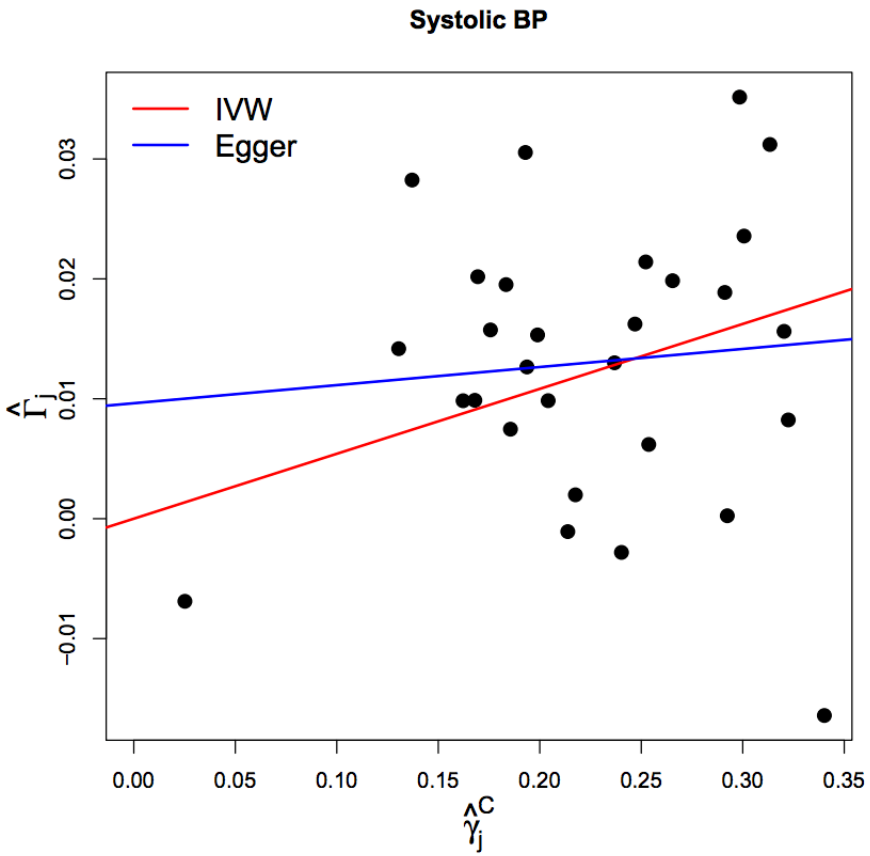
FUNNEL PLOTS

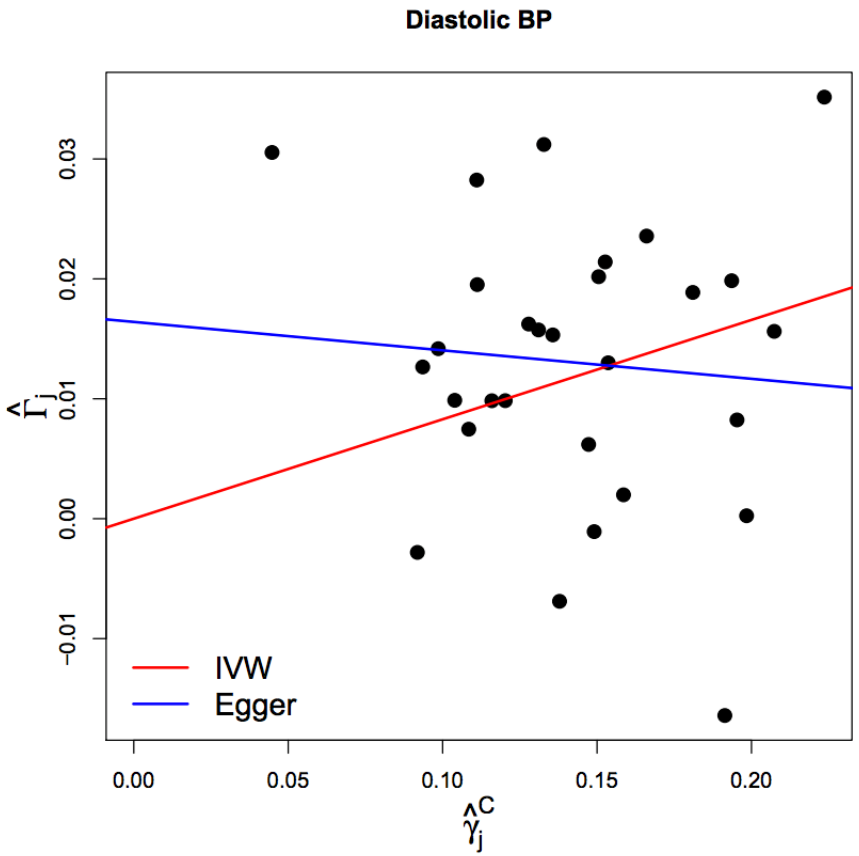




BP and Coronary Disease

Scatter Plots



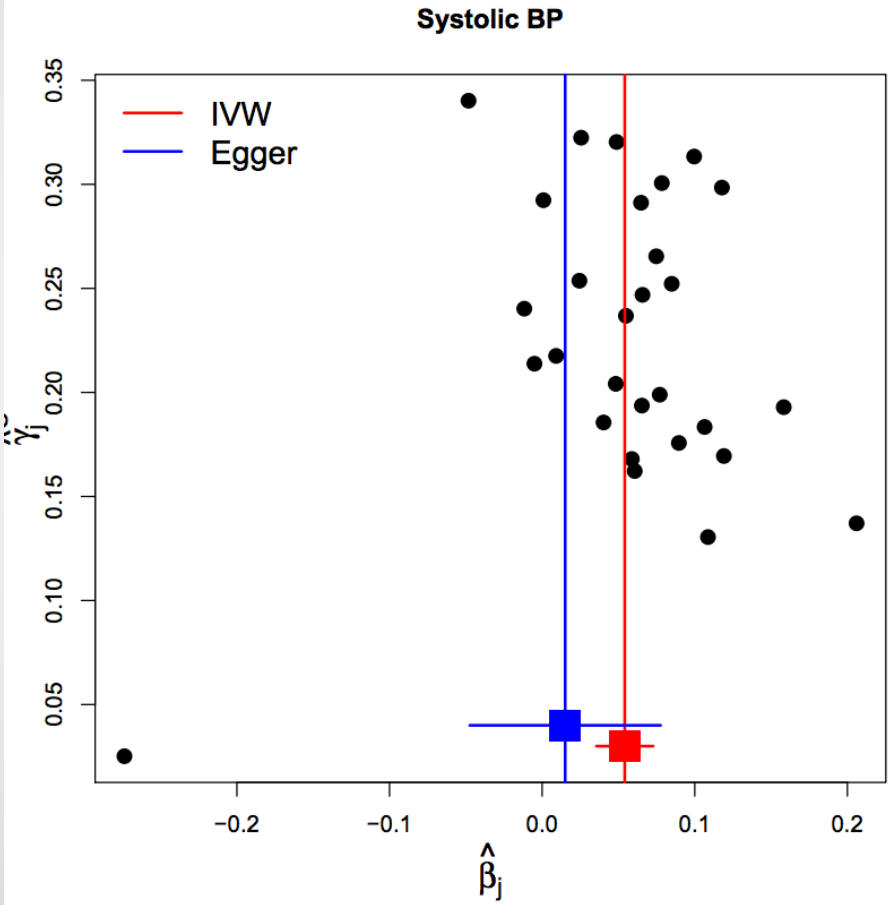


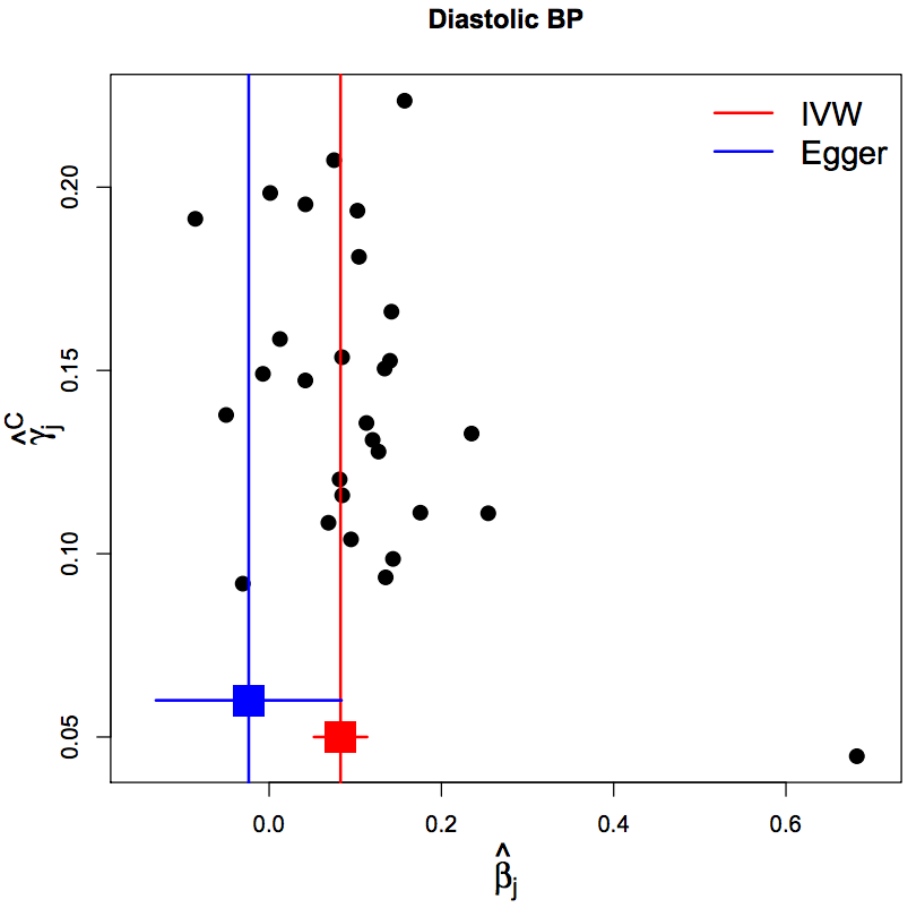
Egger test for intercept p=0.2

Egger test for intercept p=0.054

BP and Coronary Disease

FUNNEL PLOTS





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

Weighted Median Approach

Simple Median Method

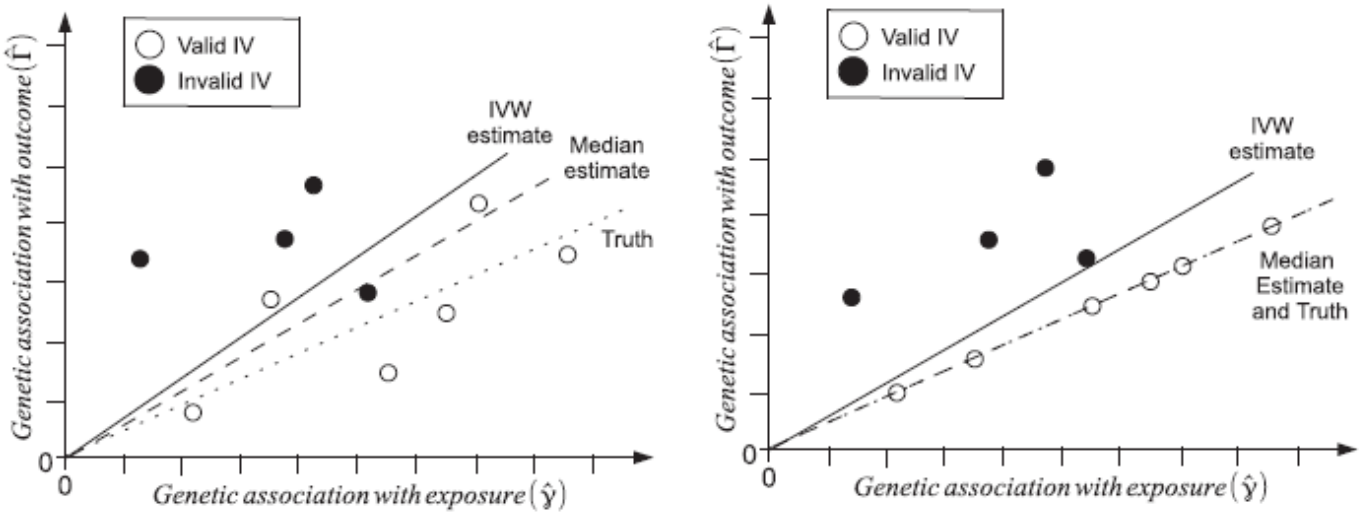


Figure 2. Fictional example of a Mendelian randomization analysis with 10 genetic variants—six valid instrumental variables (hollow circles) and four invalid instrumental variables (solid circles) for finite sample size (left) and infinite sample size (right) showing IVW (solid line) and simple median (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

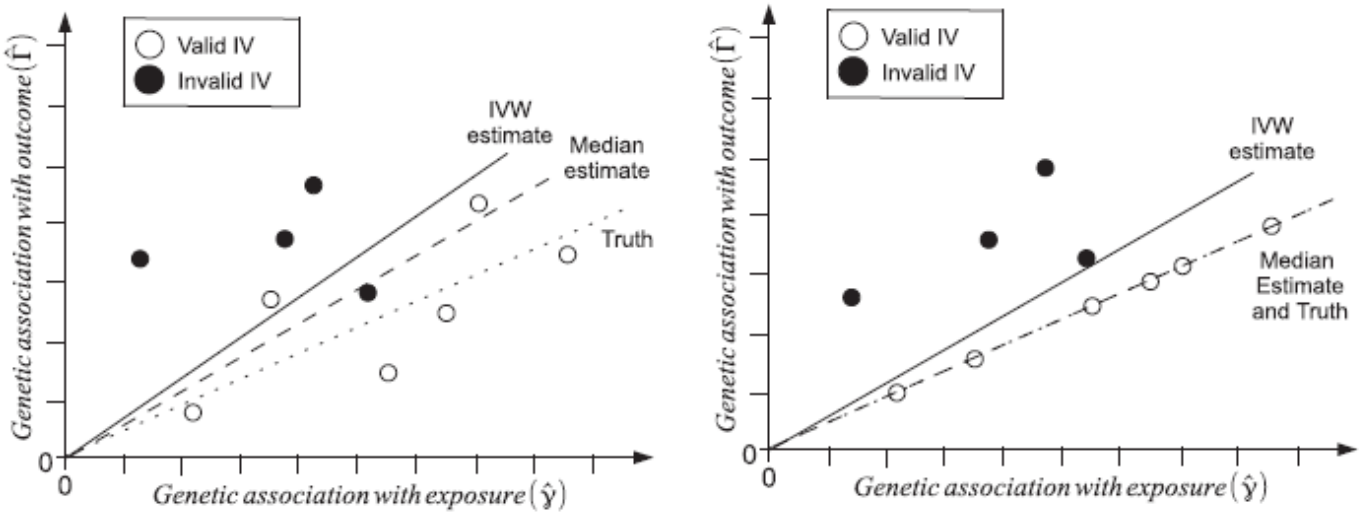
Table 1. Weights and percentiles of weighted median function

	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$	$\hat{\beta}_4$	$\hat{\beta}_5$	$\hat{\beta}_6$	$\hat{\beta}_7$	$\hat{\beta}_8$	$\hat{\beta}_9$	$\hat{\beta}_{10}$
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.

$$w_j' = \frac{\hat{\gamma}_j^2}{\sigma^2_{\gamma_j}} \qquad w_j = \frac{w_j'}{\Sigma_j w_j'}$$

Penalized Weighted Median Method



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Although the invalid IVs do not contribute directly to the median estimate, they do influence it in small samples

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

Penalized Weighted Median Method

- One way of minimizing this problem is down-weighting the contribution to the analysis of genetic variants with heterogeneous ratio estimates

- Heterogeneity between estimates can be quantified by Cochrane's Q statistic:

$$Q = \Sigma_j Q_j = \Sigma_j w_j' (\hat{\beta}_j - \hat{\beta})^2$$

- The Q statistic has a chi-squared distribution on J – 1 degrees of freedom under the null hypothesis of no heterogeneity

- Each individual component of Q has a chi-square distribution with 1 df. Bowden proposes using a one sided upper P value (denoted q_j):

$$w_j^* = w'_j \times min(1, 20q_j)$$

Penalized 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.

Mode Based Estimator

ZEMPA

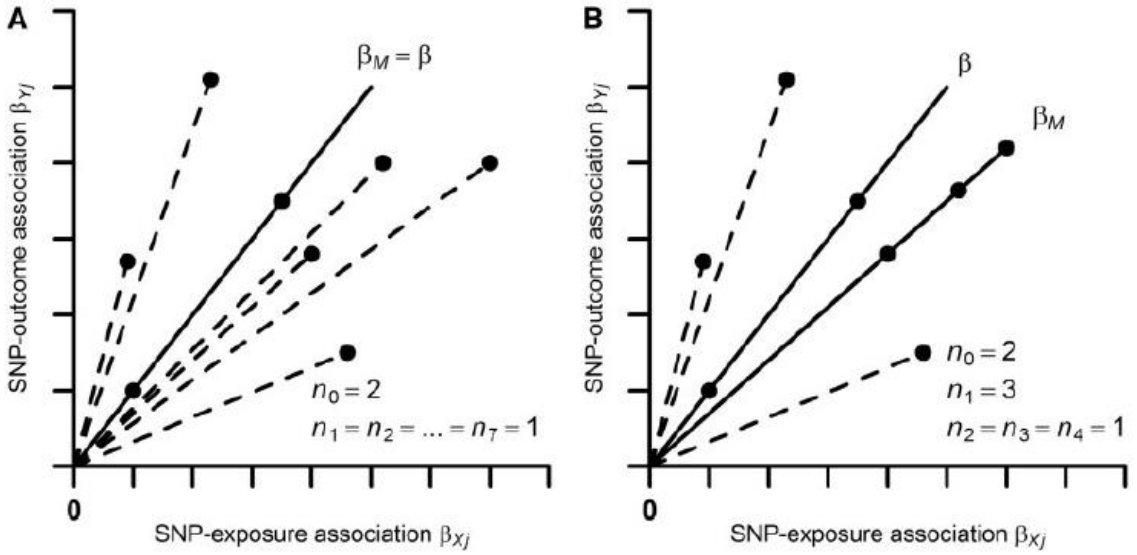
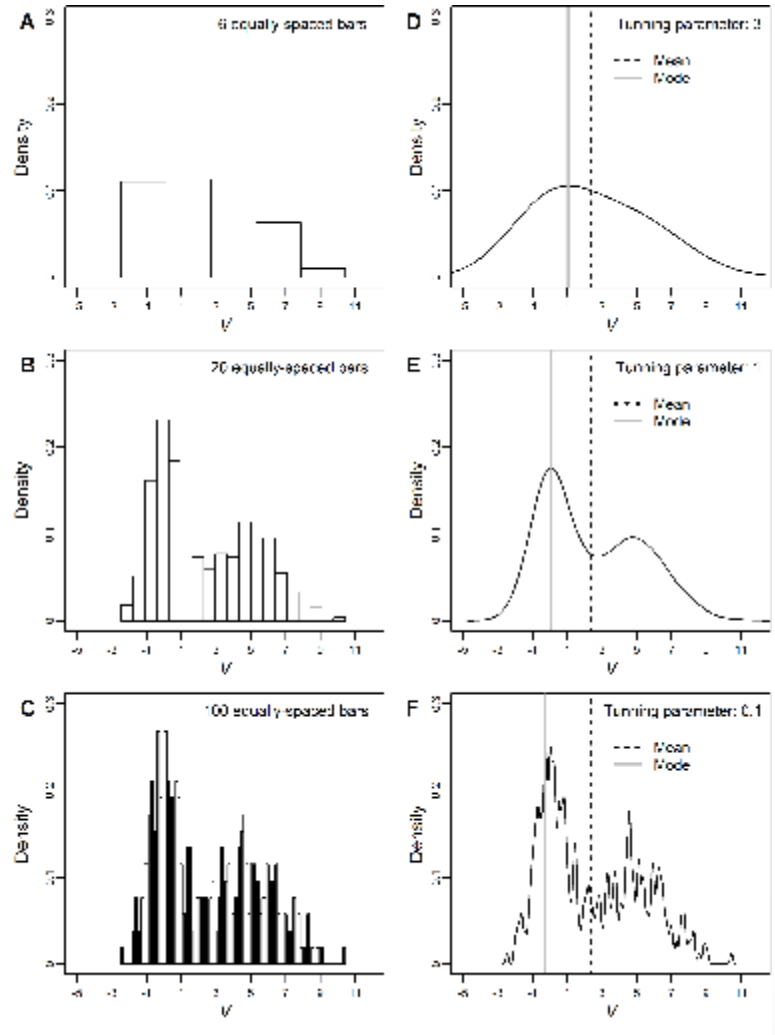
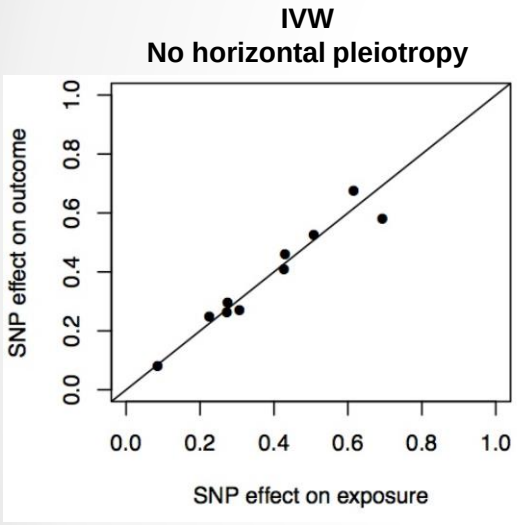


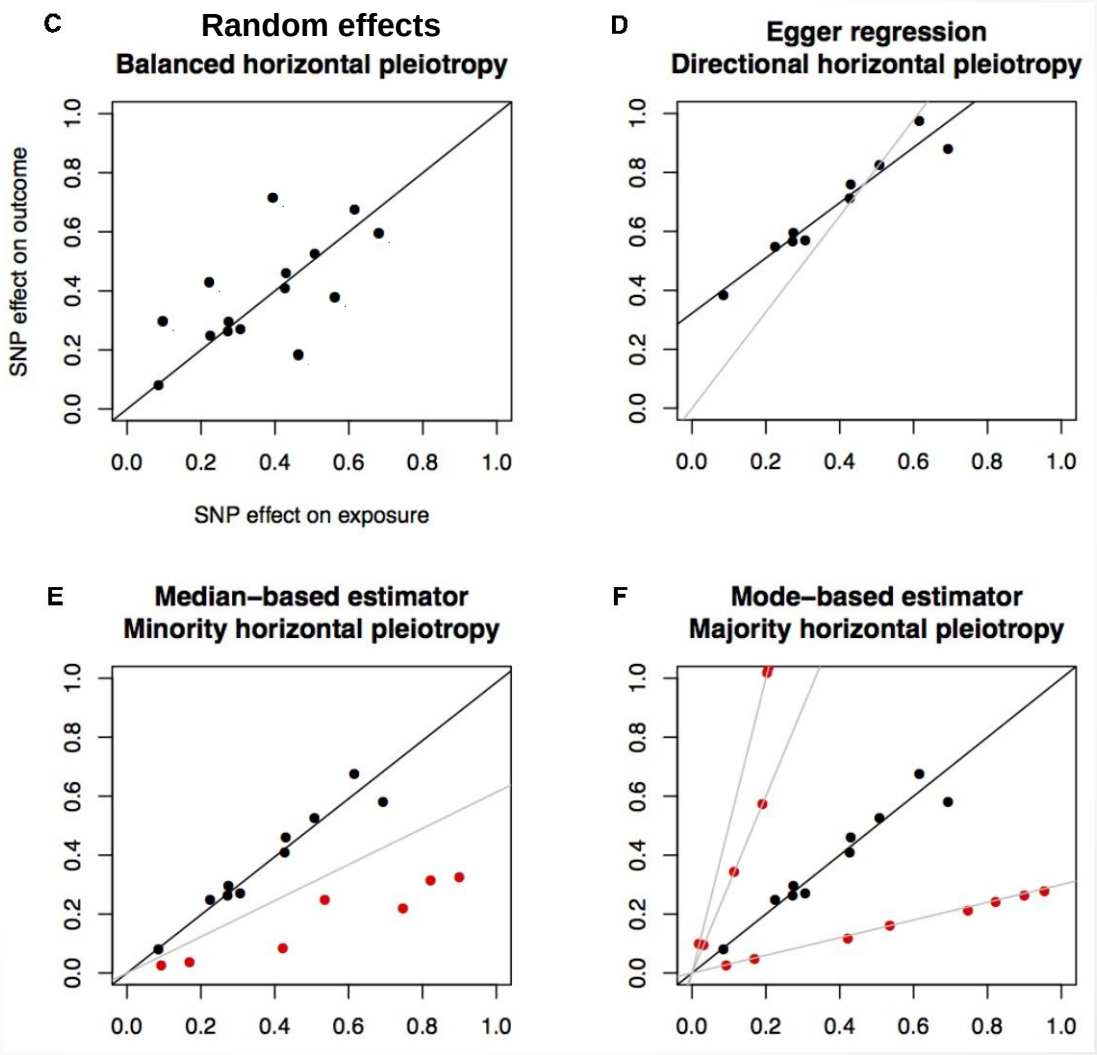
Figure 1. Illustration of the ZEro Modal Pleiotropy Assumption (ZEMPA) in the simple (i.e. unweighted) mode-based estimate (MBE). β_M is the simple MBE causal effect and β is the true causal effect; n_l denotes the number of variants with a given horizontal pleiotropic effect (n_0 denotes the number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

Kernel Density Estimation



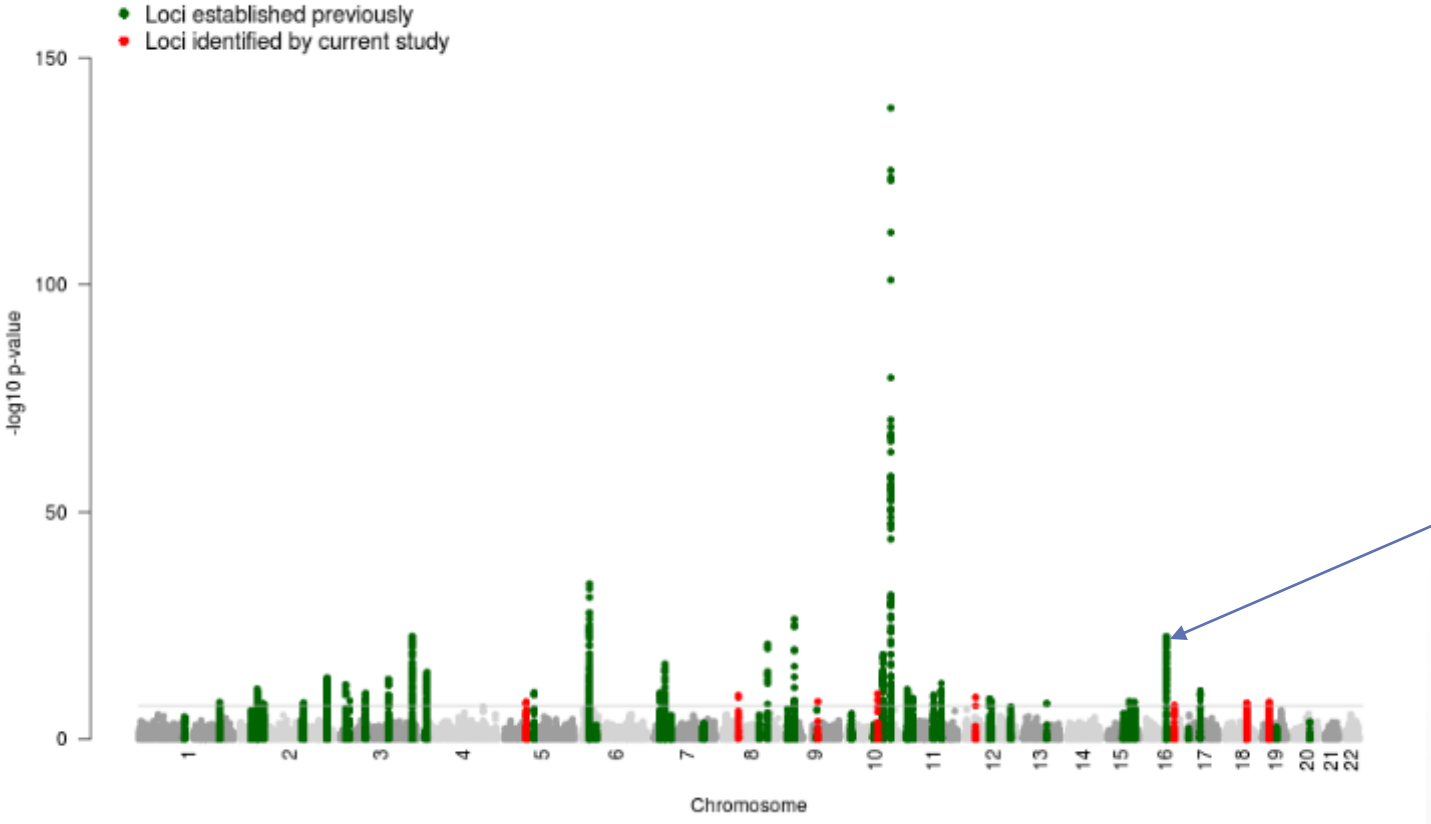
Summary of Robust Estimators



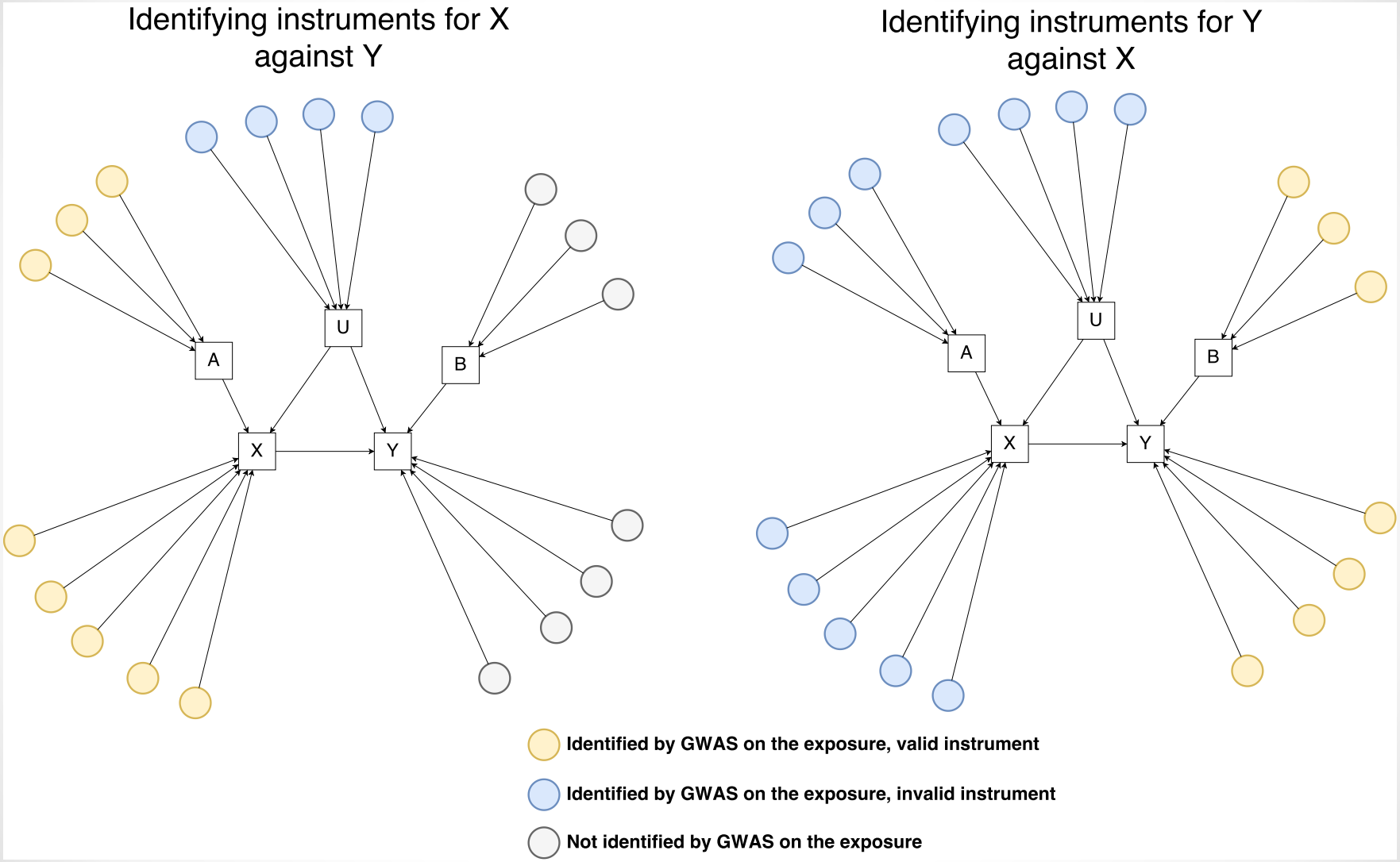


Reverse causal instruments

Problem: MR of type 2 diabetes on BMI

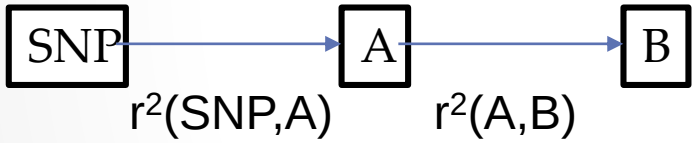


GWAS of T2D reveals *FTO* variant - Famously associated with BMI


Identifying instruments for X against Y
A
U
B
X
Y
Identifying instruments for Y against X
A
U
B
X
Y
Identified by GWAS on the exposure, valid instrument
Identified by GWAS on the exposure, invalid instrument
Not identified by GWAS on the exposure

Can we avoid including reverse-causal SNPs as instruments?

- If A causes B and B causes C
- The effect of A on B should be larger than the effect of A on C



Expect that

$r^2(SNP,B) = r^2(SNP,A) \times r^2(SNP,B)$

Steiger test used to evaluate if $r^2(SNP,A) > r^2(SNP,B)$

If this is not satisfied, infer that this instrument is not influencing the exposure primarily.

Summary

- MR uses natural randomization to mimic an RCT
- It is useful, data is abundant, but it is not a panacea for causal inference
- Often valuable for proving that an hypothesised association is not causal
- Crucial to perform sensitivity analyses and obtain metrics regarding the likely reliability of the MR estimates

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