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

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

$$w_i = \frac{1}{\mathrm{var}(\hat{\beta}_i)}$$

$$\hat{\beta}_{pooled} = \frac{\sum_{i=1}^{N}(w_i * \hat{\beta}_i)}{\sum_{i=1}^{N}(w_i)}$$

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

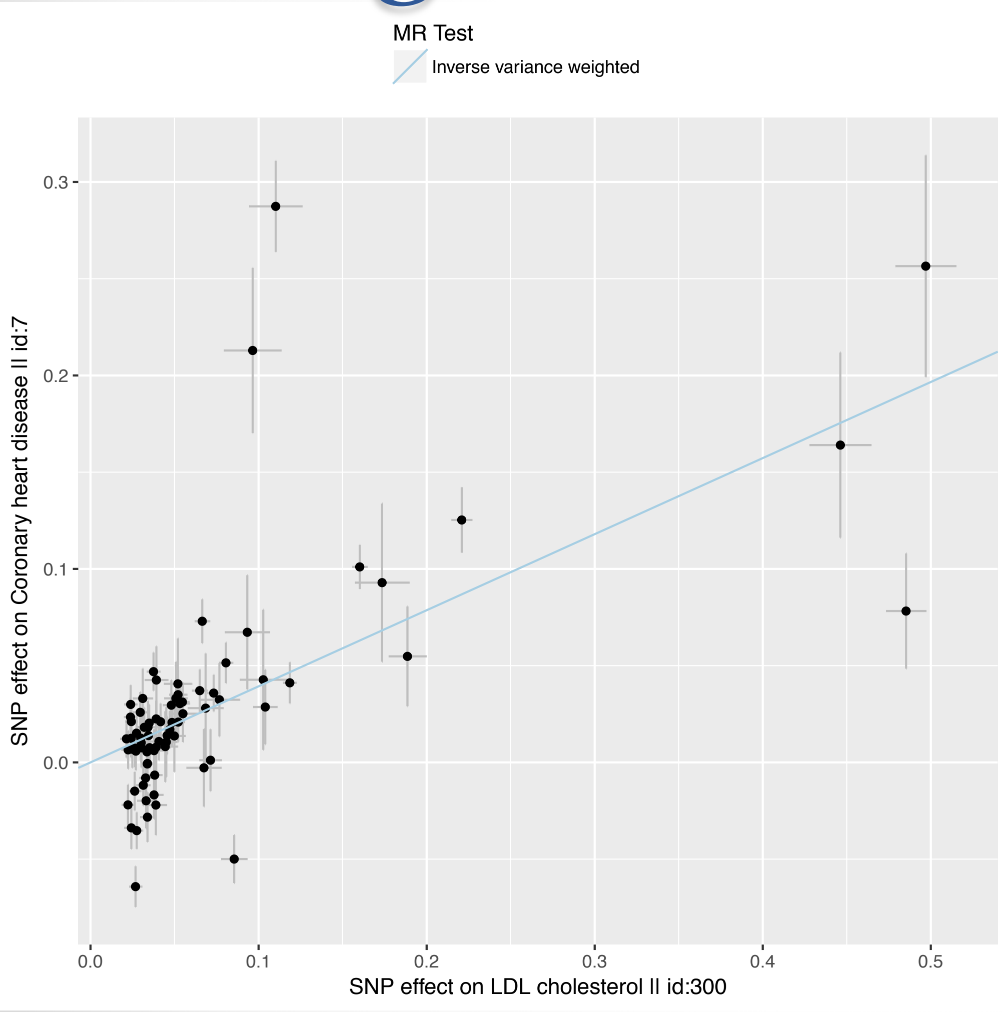
For N studies, each study *i* contributes more to the meta analysis if its standard error is lower

Calculate p-value

$$\chi^2_{df=1} = \frac{\hat{\beta}_{pooled}^{\;2}}{se_{pooled}^{\;2}} = \frac{\left(\sum_{i=1}^{N} w_i * \hat{\beta}_i\right)^2}{\sum_{i=1}^{N} w_i}$$

$$z = \frac{\hat{\beta}_{pooled}}{se_{pooled}} = \frac{\sum_{i=1}^{N} w_i * \hat{\beta}_i}{\sqrt{\sum_{i=1}^{N} w_i}}$$

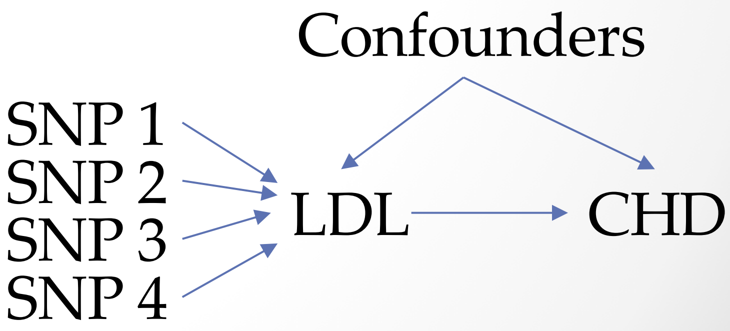
Fixed Effects IVW-MR and Weighted Linear regression



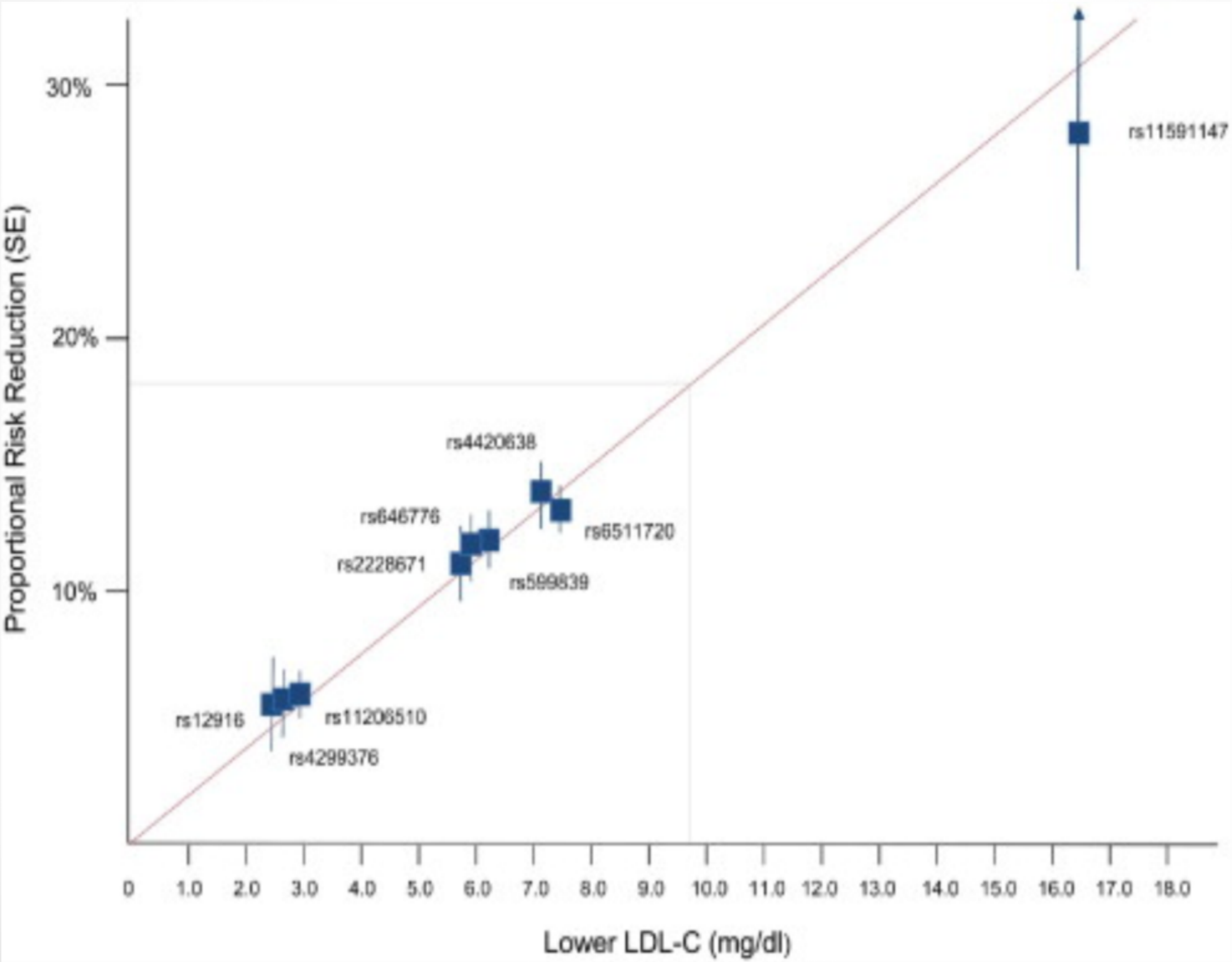
IVW is equivalent to a weighted regression of SNP-outcome effects on SNP-exposure effects passing through the origin

The weights are 1/SE_SNP_outcome

The slope is the estimate of the causal effect



LDL and CHD Risk



Ference et al, JACC 2012

MR methods for handling horizontal pleiotropy

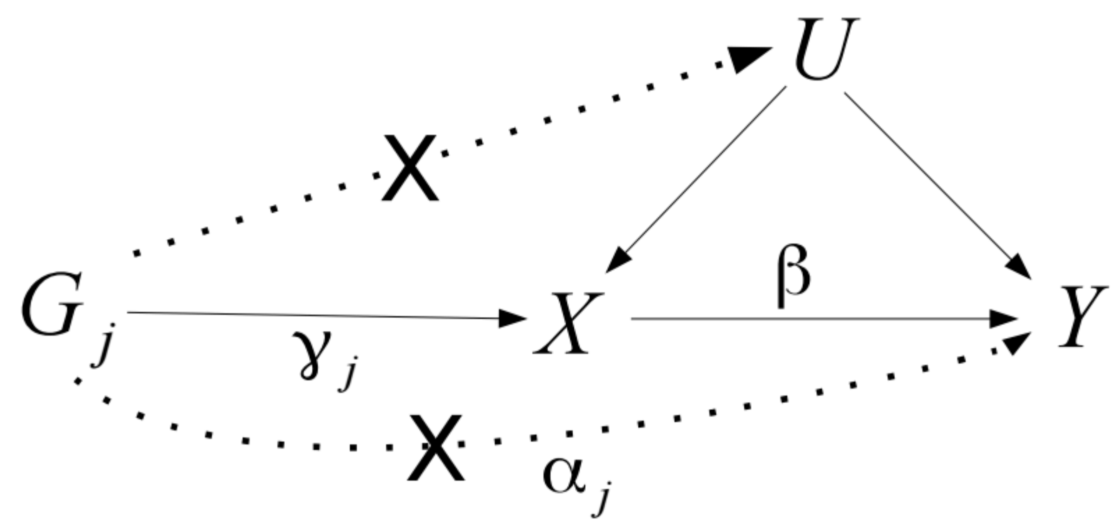
Many methods now exist

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



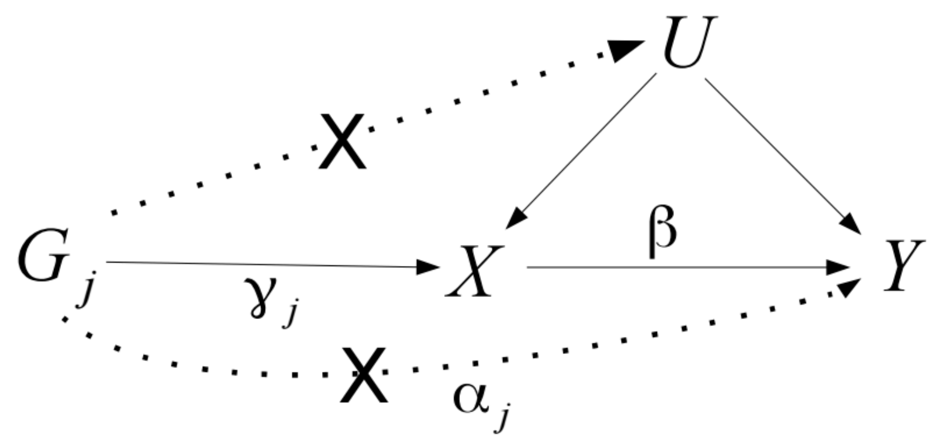
Wald = $\frac{\text{Beta-GY}}{\text{Beta-GX}}$

Causal estimate using Wald method:

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

Two Sample MR:

Multiple Variants



Causal estimate using IVW from summarised data:

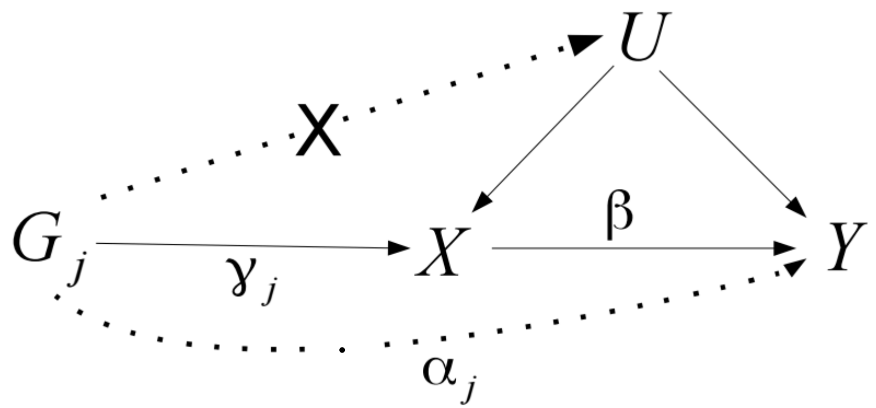
(Approximates TSLS)

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

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

$$Y_i = \Gamma_j G_{ij} + \epsilon'^{Y}_{ij}$$
$$= (\alpha_j + \beta\gamma_j) G_{ij} + \epsilon'^{Y}_{ij}.$$



Single variant Wald estimate:

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

Multiple variant
TSLS / IVW :

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

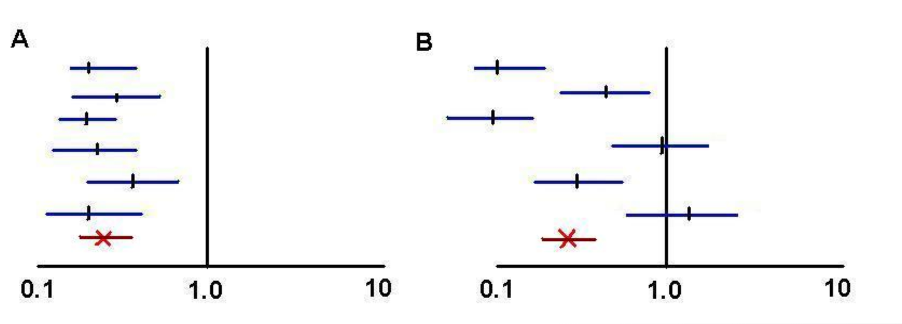
Heterogeneity

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

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



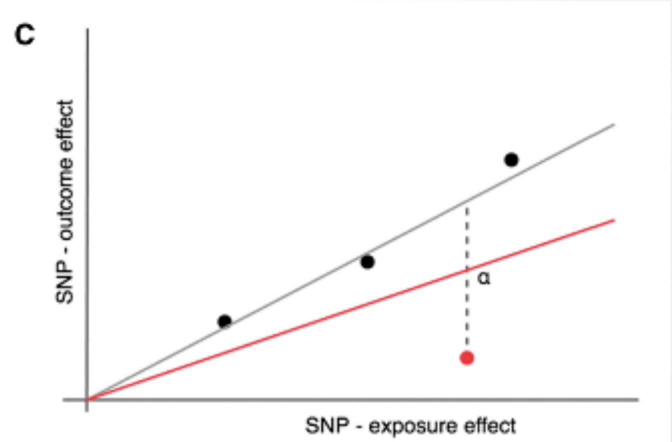
n=6 instruments
Expect Q = 5 if there is no heterogeneity
Q is chi-square distributed with n-1 degrees of freedom

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

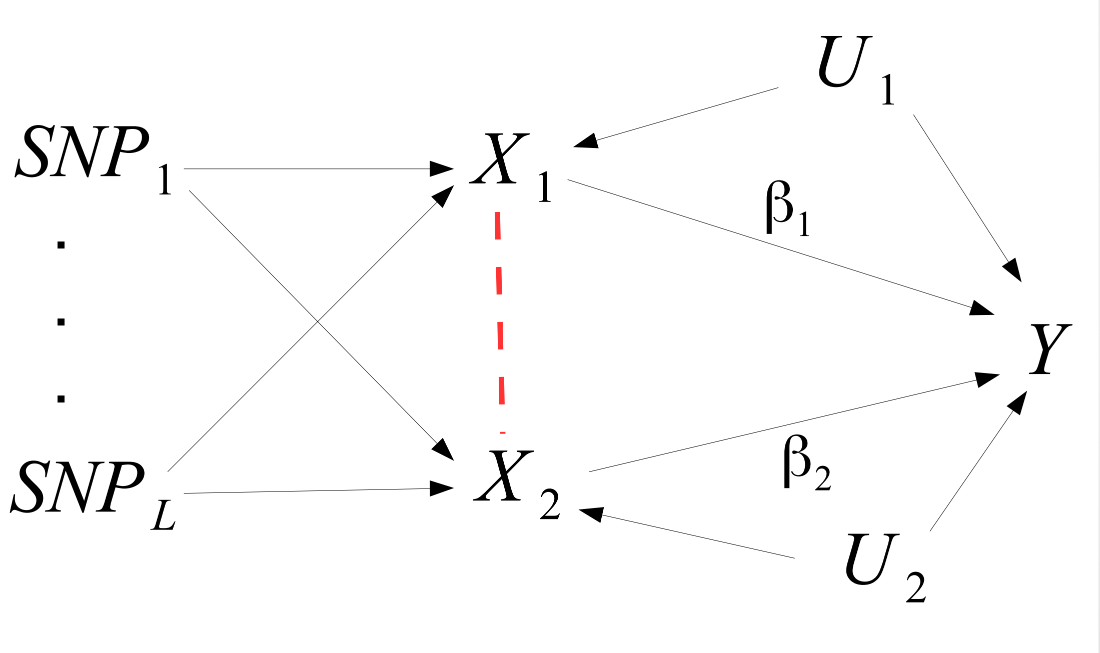
However – beware of:

- 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

MR Egger Regression

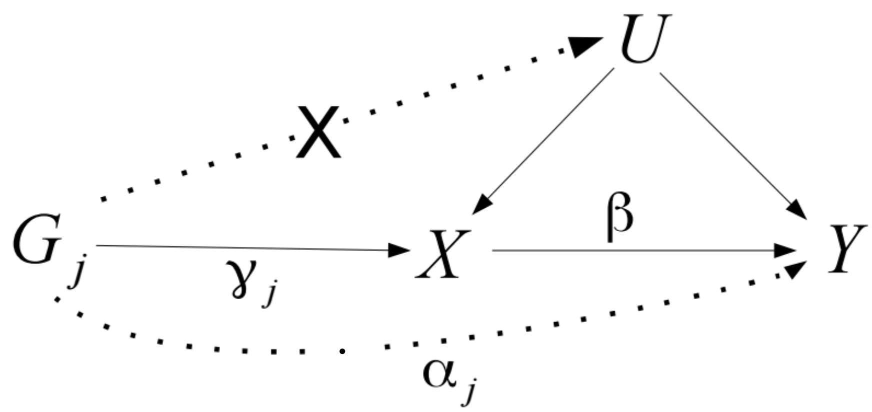
MR Egger Regression: Central concept

- 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

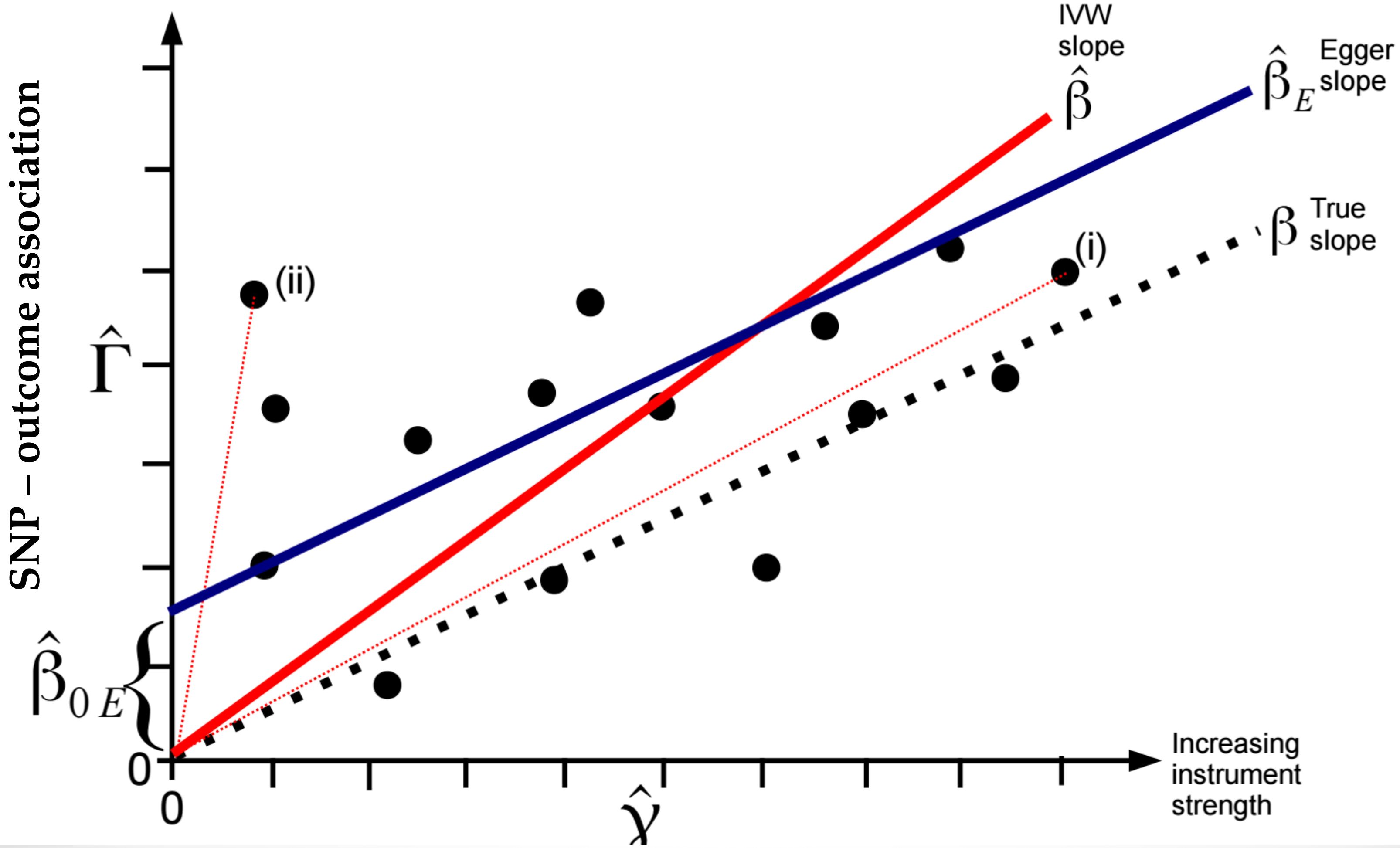
$$
\begin{aligned}
Y_i &= \Gamma_j G_{ij} + \epsilon_{ij}^{\prime Y} \\
&= (\alpha_j + \beta\gamma_j) G_{ij} + \epsilon_{ij}^{\prime Y}.
\end{aligned}
$$



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:

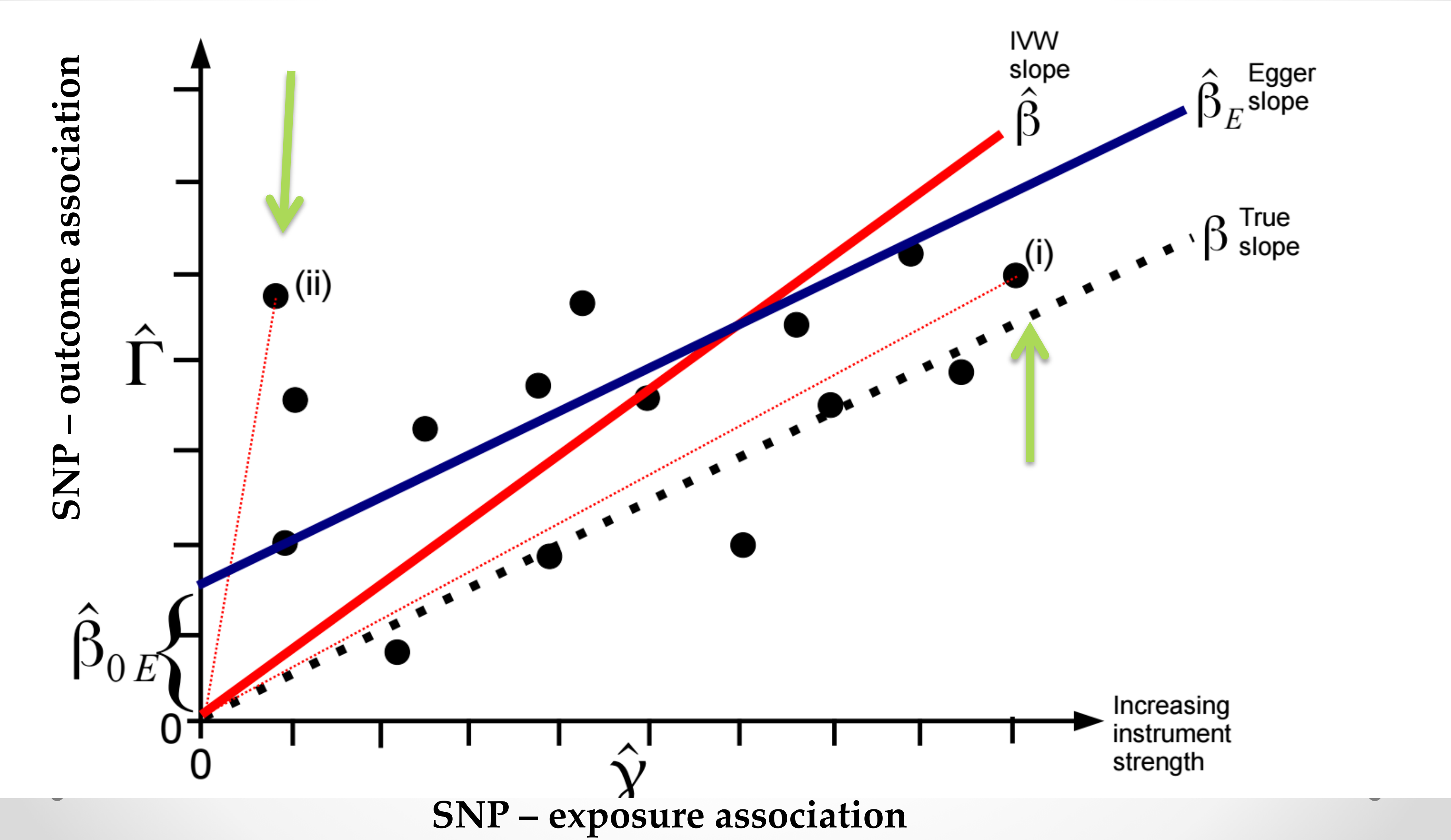
ALL INVALID INSTRUMENTS
INSIDE ASSUMPTION SATISFIED



SNP – exposure association

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

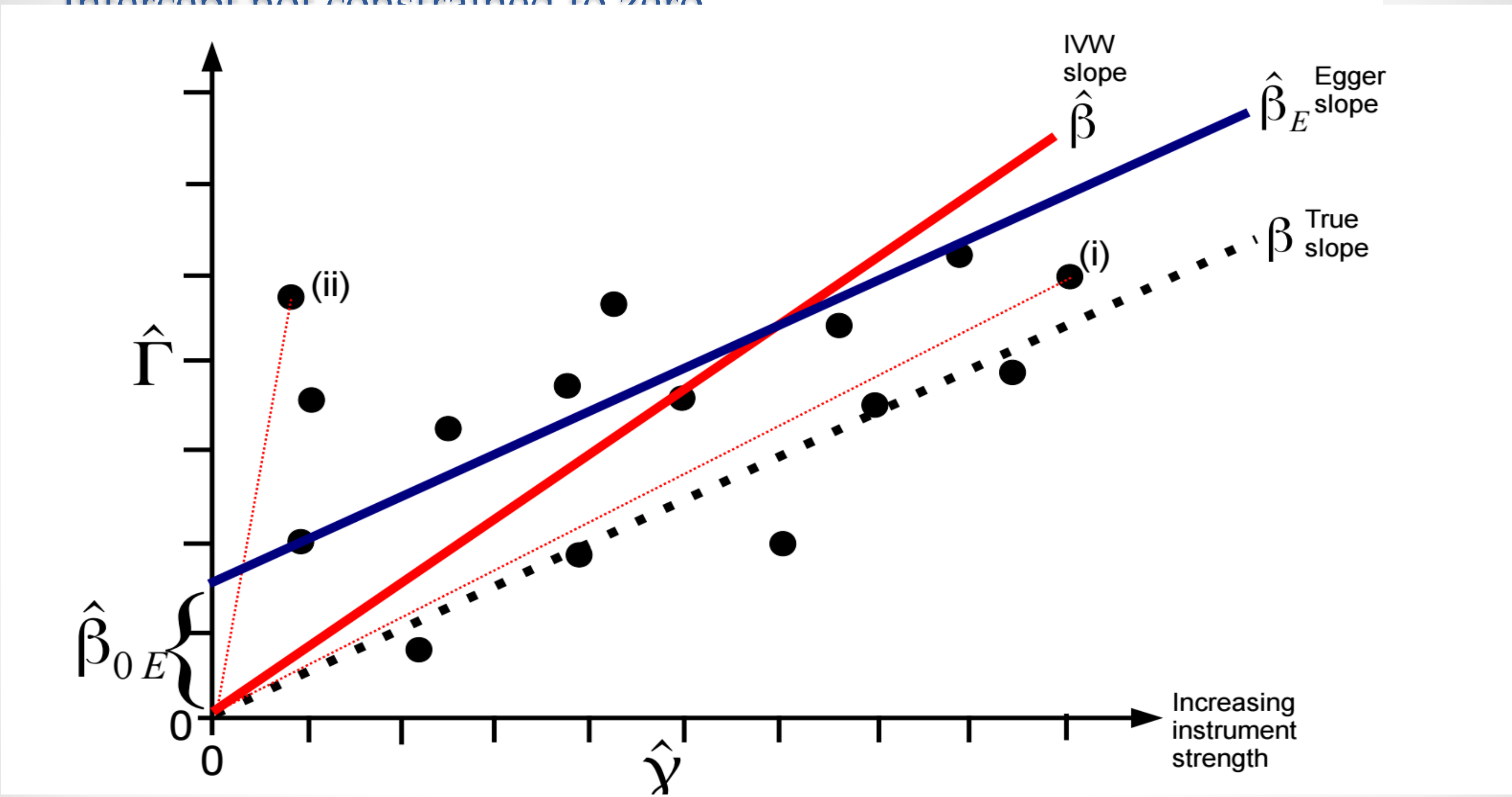
Bias of ratio estimator $\hat{\beta}_j = \frac{\hat{\Gamma}_j}{\hat{\gamma}_j}$ is inversely proportional to γ_j.



Egger regression:

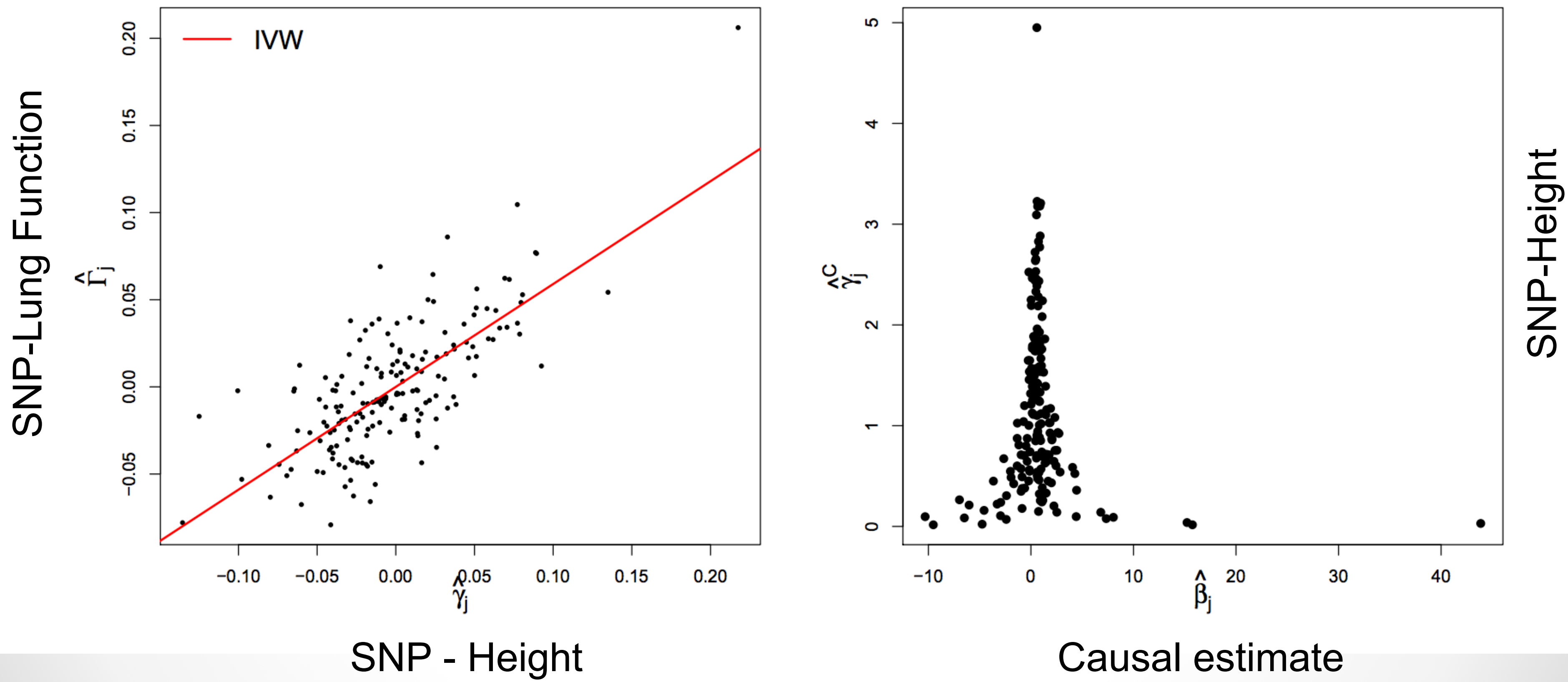
$$\hat{\Gamma}_j = \beta_{0E} + \beta_E \hat{\gamma}_j.$$

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

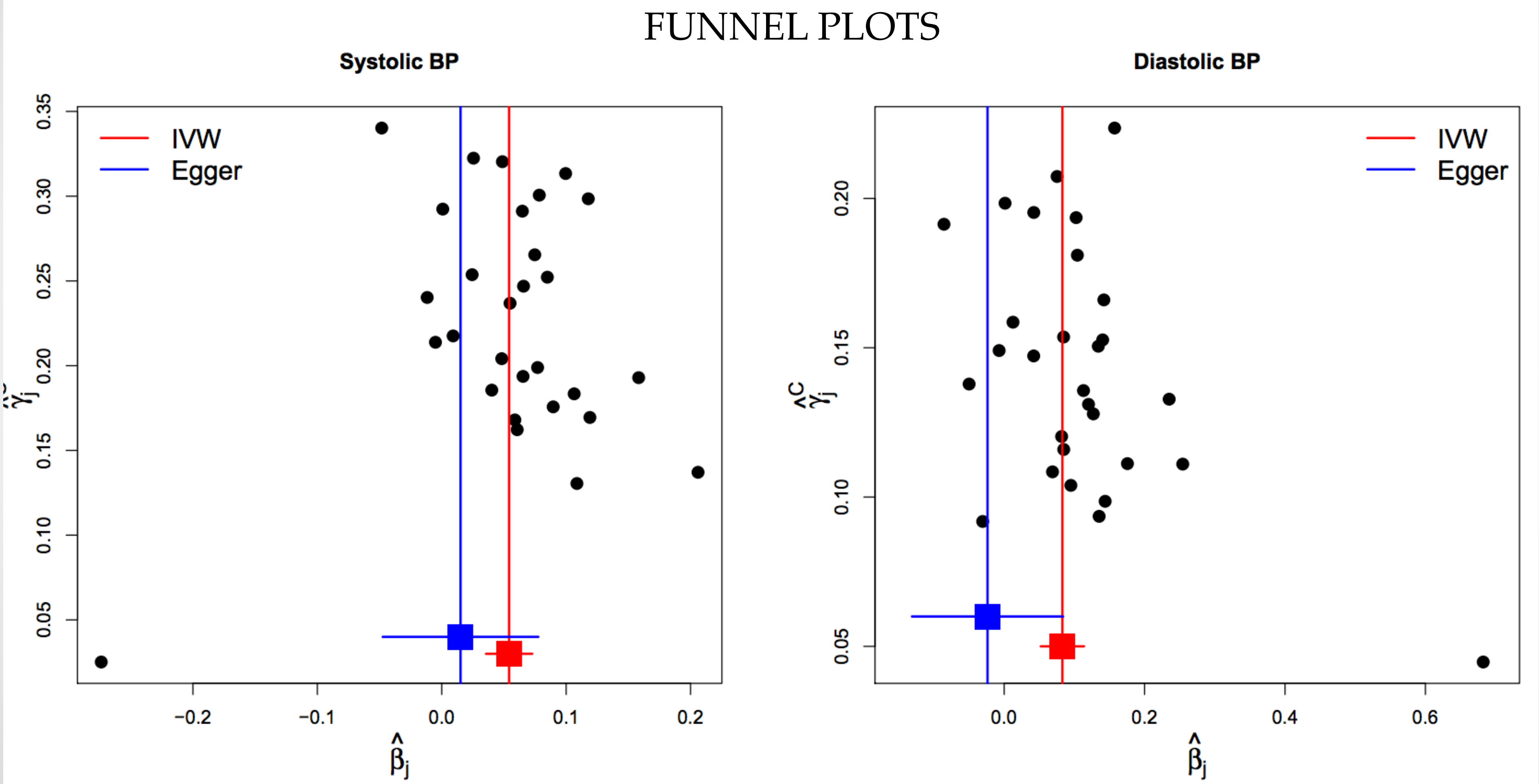
Height and lung function



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

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

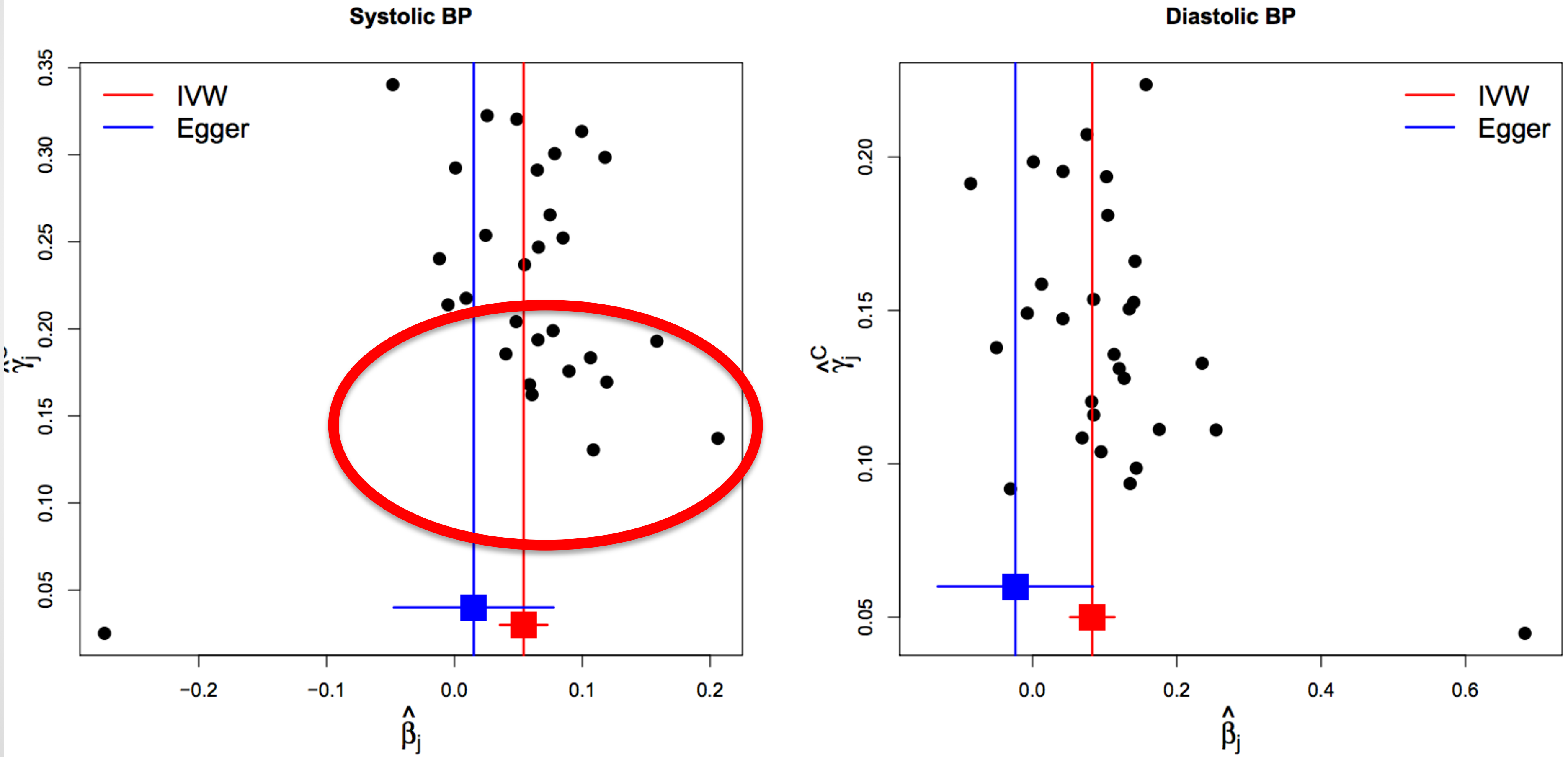
BP and Coronary Disease



Visual evidence for asymmetry

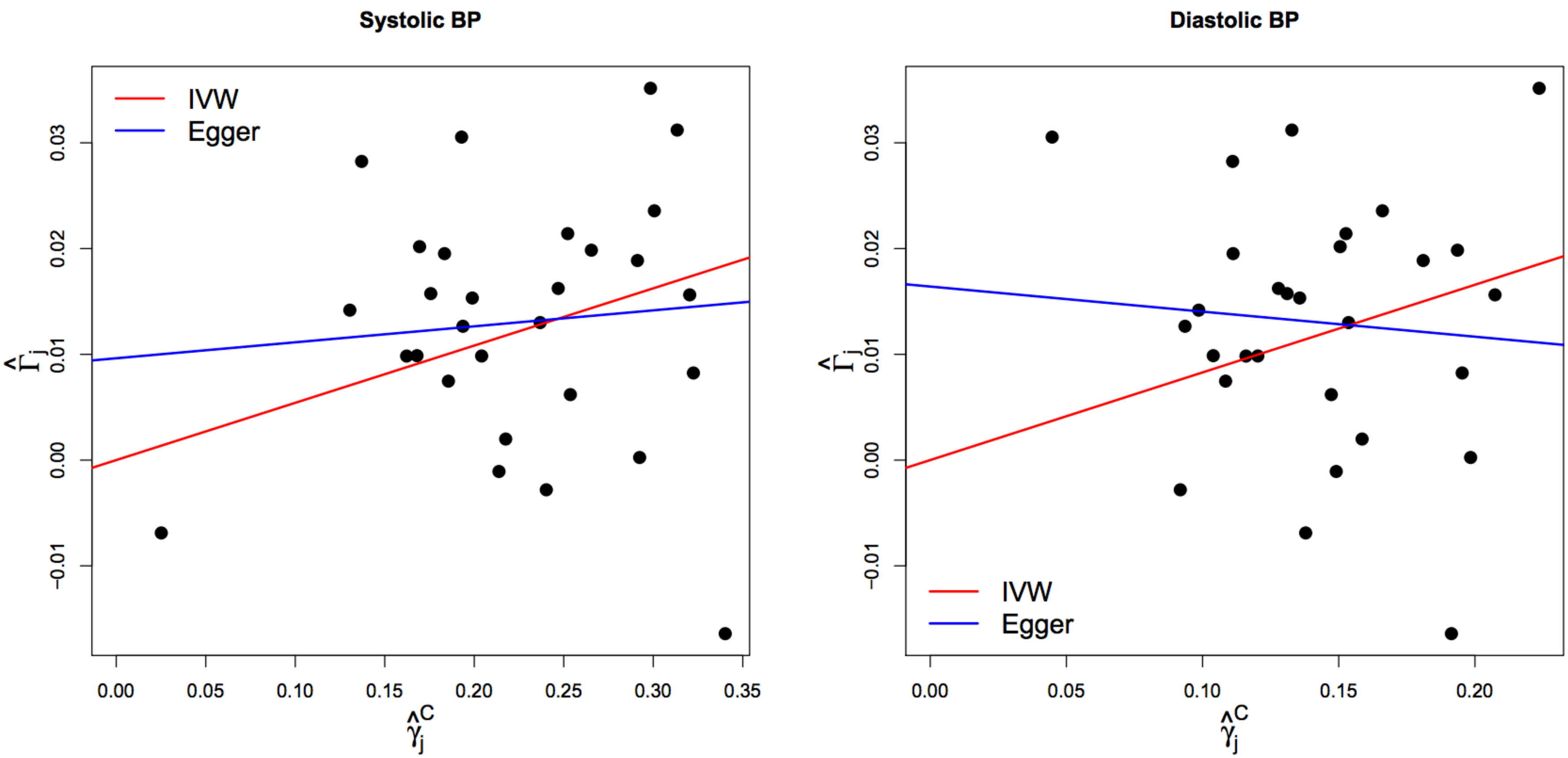
BP and Coronary Disease

FUNNEL PLOTS



BP and Coronary Disease

Scatter Plots

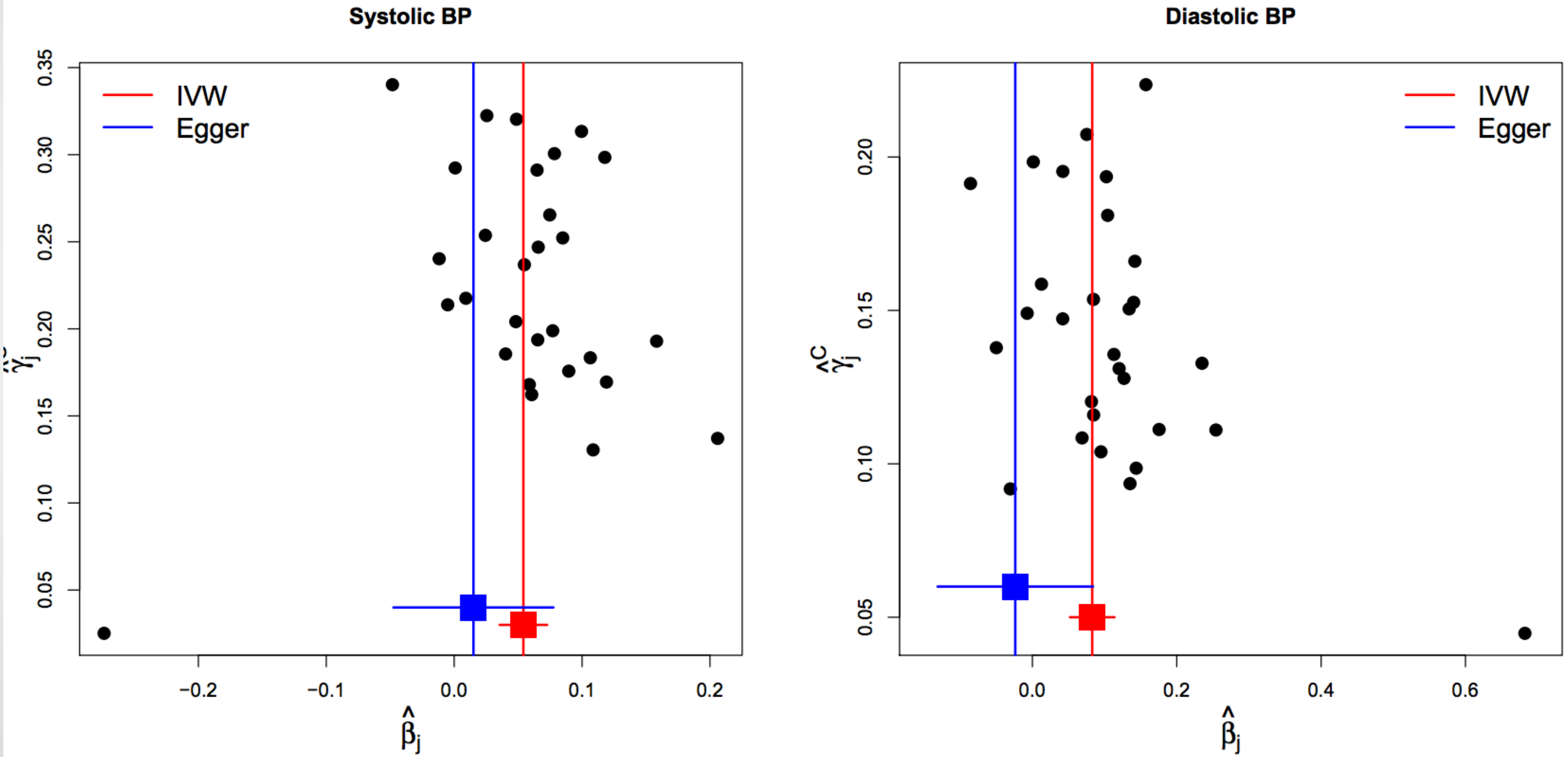


Egger test for intercept p=0.2

Egger test for intercept p=0.054

BP and Coronary Disease

FUNNEL PLOTS



IVW= 0.054 logOR/mmHg $p=4\times10^{-6}$
Egger =0.015 logOR/mmHg p=0.6

IVW= 0.083 logOR/mmHg $p=1\times10^{-5}$
Egger =-0.024 logOR/mmHg p=0.7

Weighted Median Approach

Simple Median Method

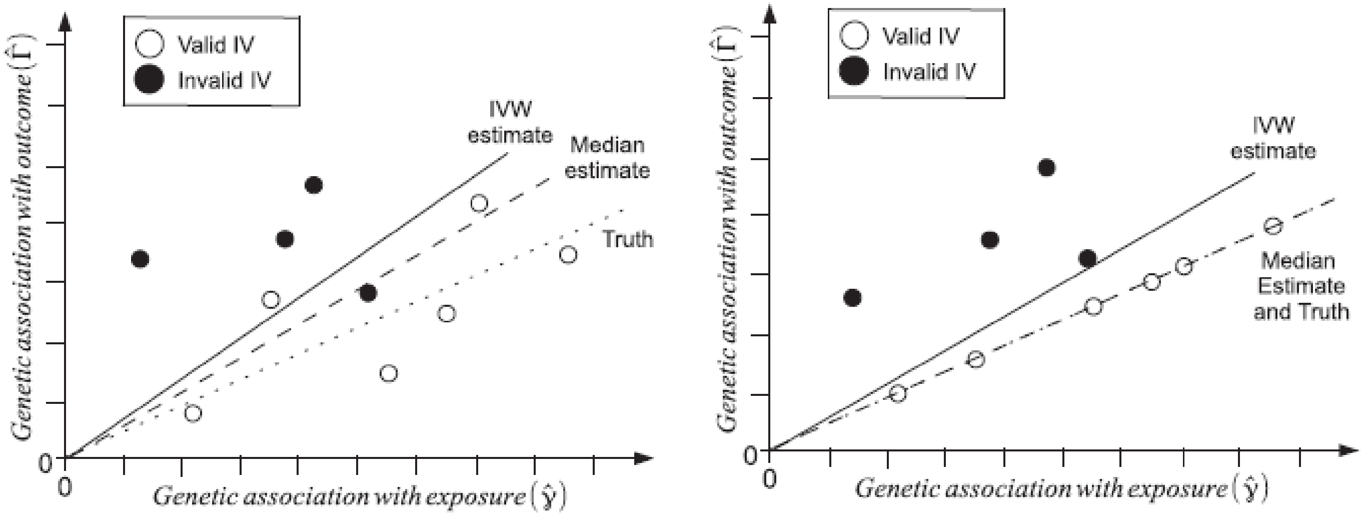


Figure 2. Fictional example of a Mendelian randomization analysis with 10 genetic variants—six valid instrumental variables (hollow circles) and four invalid instrumental variables (solid circles) for finite sample size (left) and infinite sample size (right) showing IVW (solid line) and simple median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

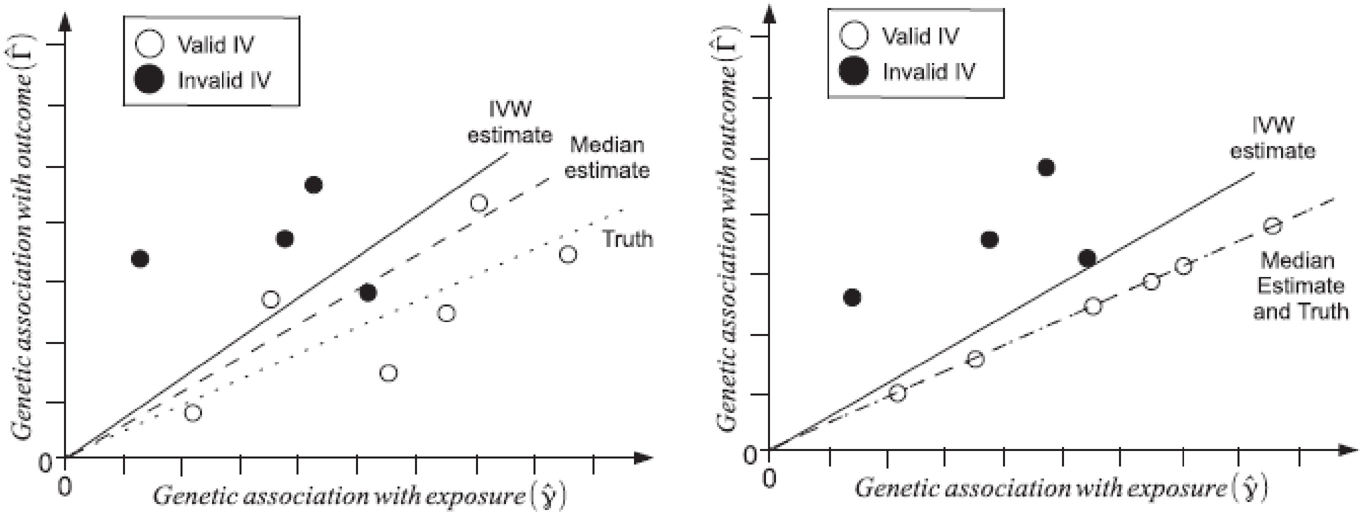
Table 1. Weights and percentiles of weighted median function

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

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

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.

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

References

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