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ARTICLES

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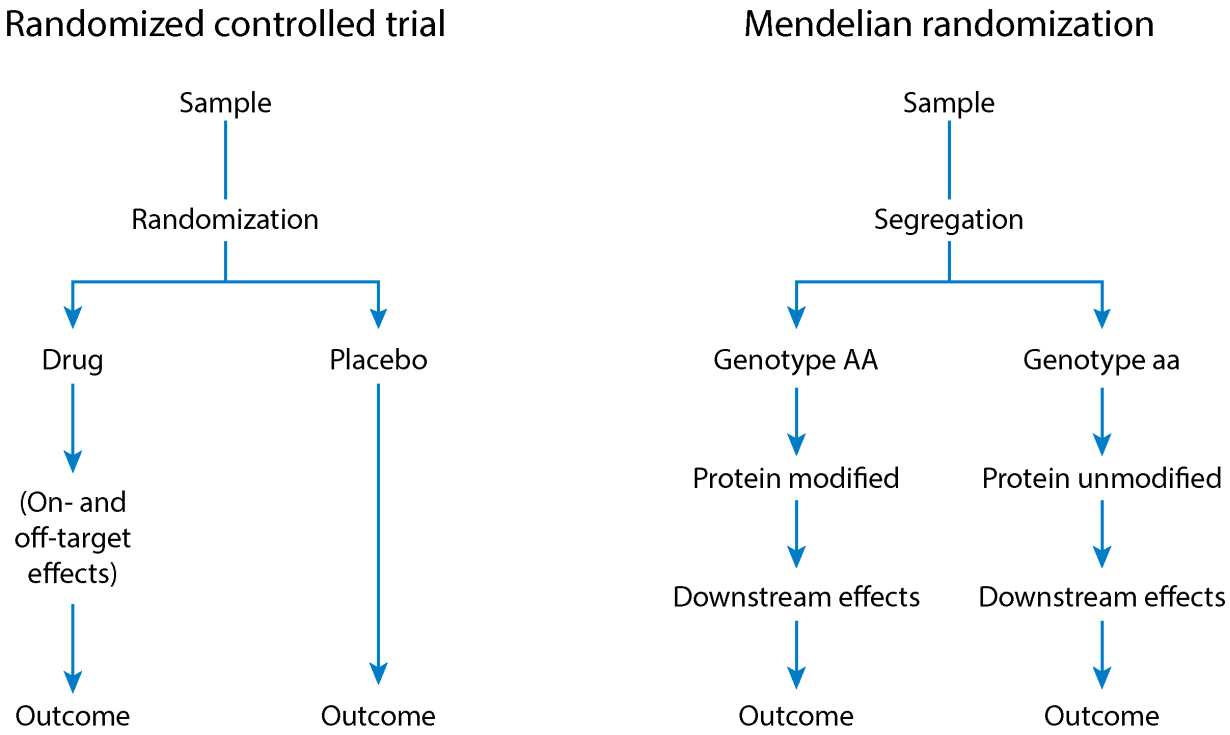
Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases

Marie Verbanck[1,2,3,7], Chia-Yen Chen[4,5,6,7], Benjamin Neale[4,5,6,8*] and Ron Do[1,2,3,8*]

Horizontal pleiotropy occurs when the variant has an effect on disease outside of its effect on the exposure in Mendelian randomization (MR). Violation of the 'no horizontal pleiotropy' assumption can cause severe bias in MR. We developed the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test to identify horizontal pleiotropic outliers in multi-instrument summary-level MR testing. We showed using simulations that the MR-PRESSO test is best suited when horizontal pleiotropy occurs in <50% of instruments. Next we applied the MR-PRESSO test, along with several other MR tests, to complex traits and diseases and found that horizontal pleiotropy (i) was detectable in over 48% of significant causal relationships in MR; (ii) introduced distortions in the causal estimates in MR that ranged on average from −131% to 201%; (iii) induced false-positive causal relationships in up to 10% of relationships; and (iv) could be corrected in some but not all instances.

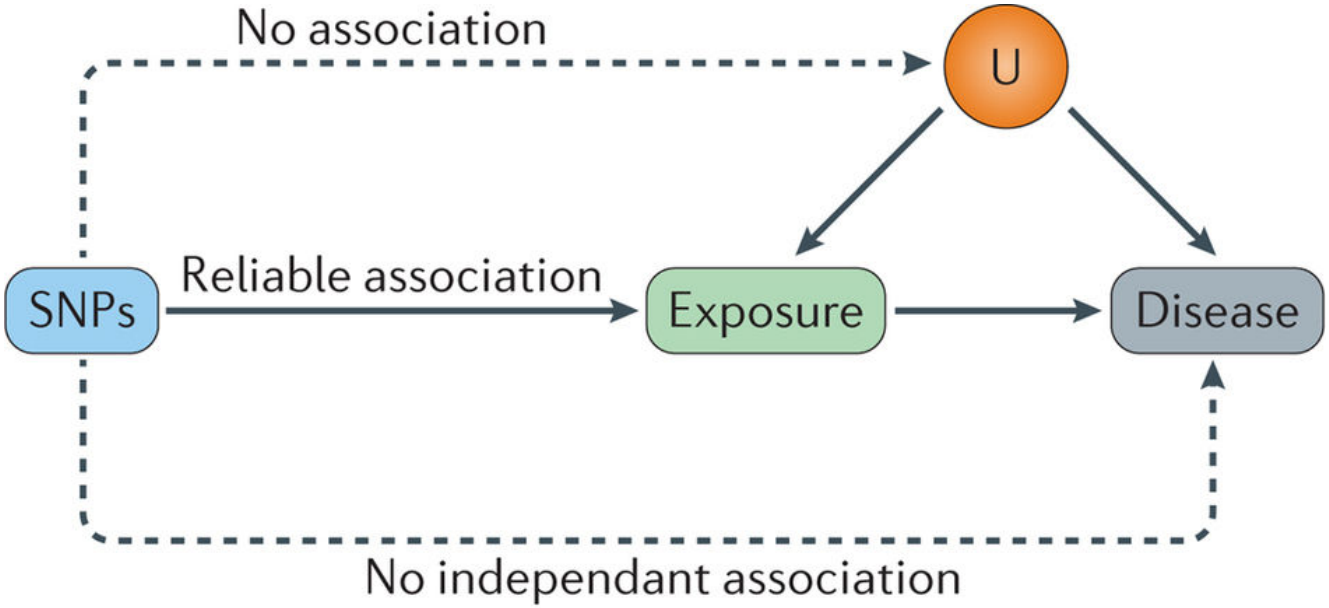
Mendelian Randomisation: rationale



Evans DM, Smith GD. 2015.
Annu. Rev. Genomics Hum. Genet. 16:327–50

Mendelian Randomization: New Applications in the Coming Age of Hypothesis-Free Causality

Mendelian Randomisation: the model



Holmes, M. V. *et al.* (2017) Mendelian randomization in cardiometabolic disease: challenges in evaluating causality *Nat. Rev. Cardiol.* doi:10.1038/nrcardio.2017.78

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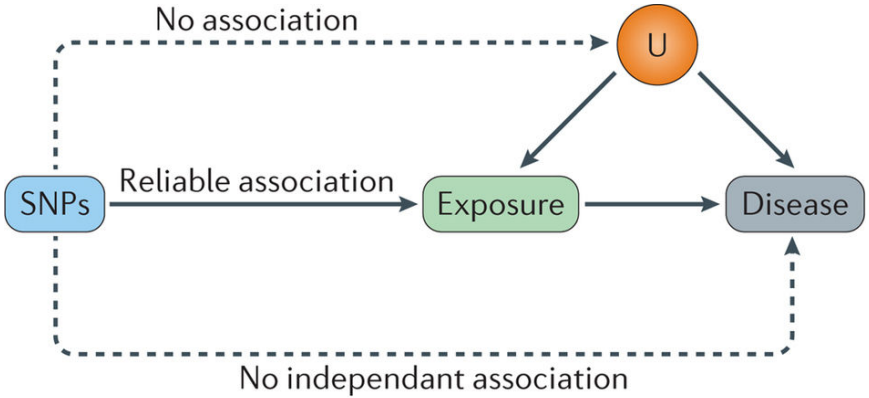
Mendelian Randomisation: some strong assumptions

The SNP (instrumental variable) is associated with the exposure

The SNP is not associated with confounders, known or unknown

All the effect of the SNP on the disease is via the exposure



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Holmes, M. V. *et al.* (2017) Mendelian randomization in cardiometabolic disease: challenges in evaluating causality *Nat. Rev. Cardiol.* doi:10.1038/nrcardio.2017.78

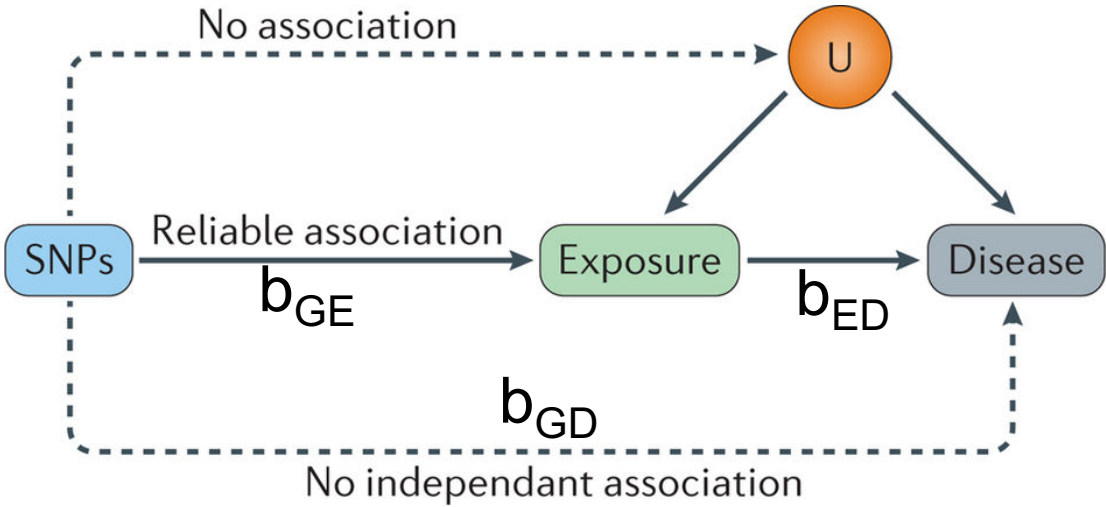
Two-Sample MR

Often we do not have large GWAS studies with SNPs, exposure and outcome

⇒ Use summary statistics of GWAS of exposure and disease: b_{GE} and b_{GD}
⇒ Extra assumption: same population ancestry
⇒ Allows bidirectional MR

Under MR assumptions:

The effect of exposure on disease for 1 SNP is $b_{ED} = b_{GD} / b_{GE}$



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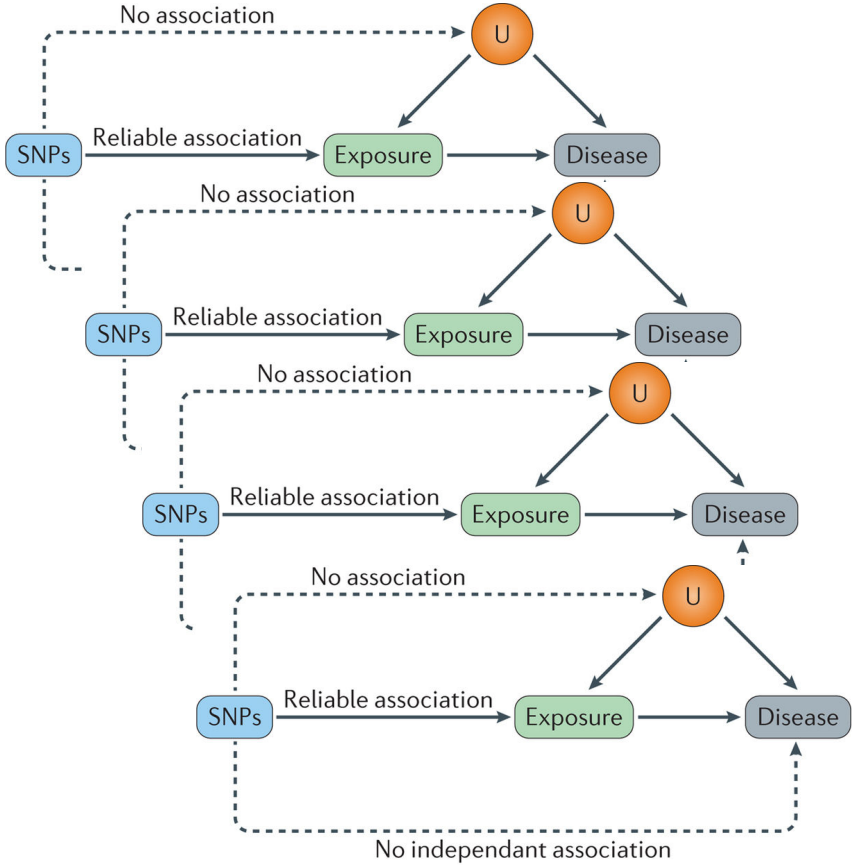
Multi Instrument MR

Integrate all the SNPs associated with the exposure

Meta-analysis approach: fixed effect, random effect, inverse variance weighted, MR Egger
http://www.mrbase.org/
Hemani et al., The MR-Base Collaboration. MR-Base: a platform for systematic causal inference across the phenome using billions of genetic associations. bioRxiv.

GSMR: Generalised Summary-data Mendelian Randomisation. Models LD between instruments and takes into account precision around betas estimated from GWAS.
http://cnsgenomics.com/software/gsmr/
Zhu et al., 2018. Causal associations between risk factors and common diseases inferred from GWAS summary data



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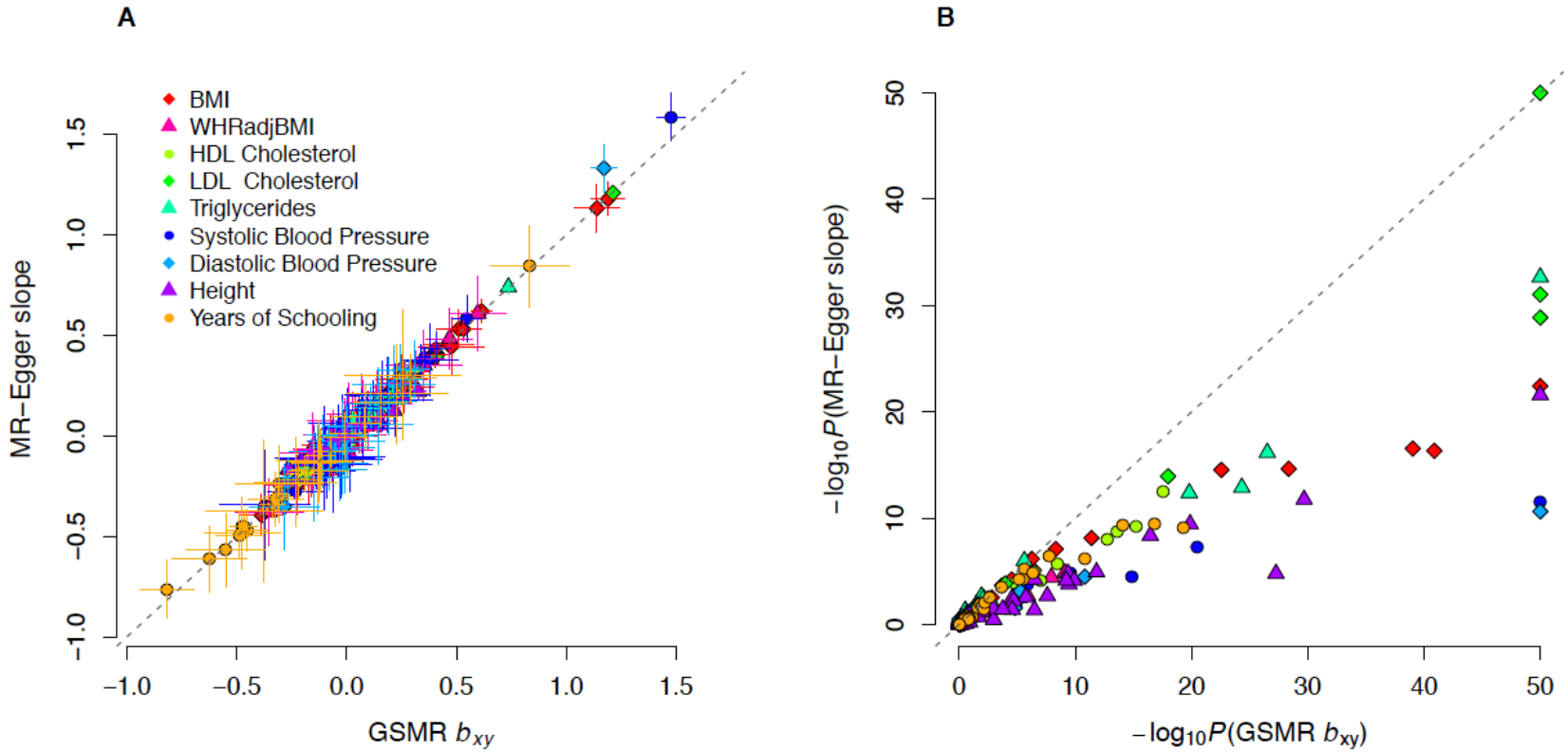
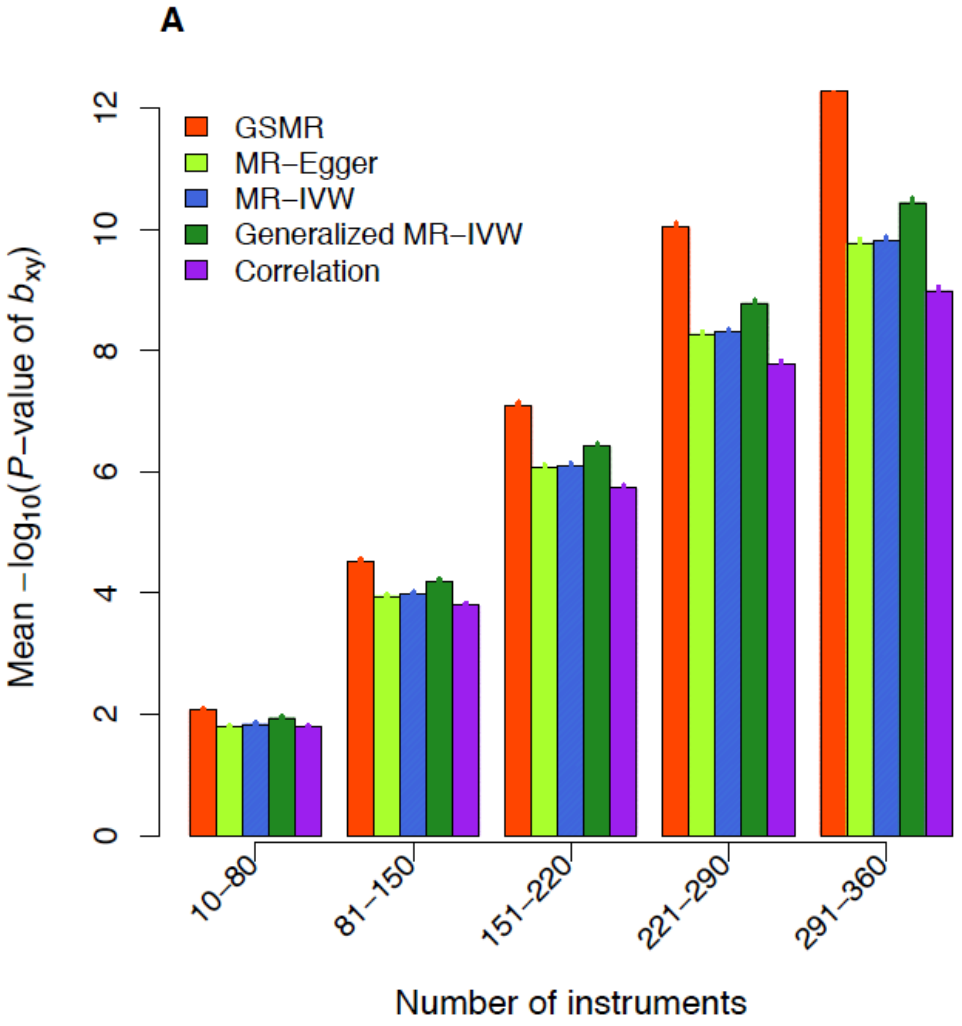
GSMR: more powerful than MR-EGGER



Zhu et al., 2018. Causal associations between risk factors and common diseases inferred from GWAS summary data

GSMR: more powerful than concurrent methods

Especially as number of instruments increases



Zhu et al., 2018. Causal associations between risk factors and common diseases inferred from GWAS summary data

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ANALYSIS

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Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets

Zhihong Zhu[1], Futao Zhang[1], Han Hu[2], Andrew Bakshi[1], Matthew R Robinson[1], Joseph E Powell[1,3], Grant W Montgomery[4], Michael E Goddard[5,6], Naomi R Wray[1], Peter M Visscher[1,7] & Jian Yang[1,7]

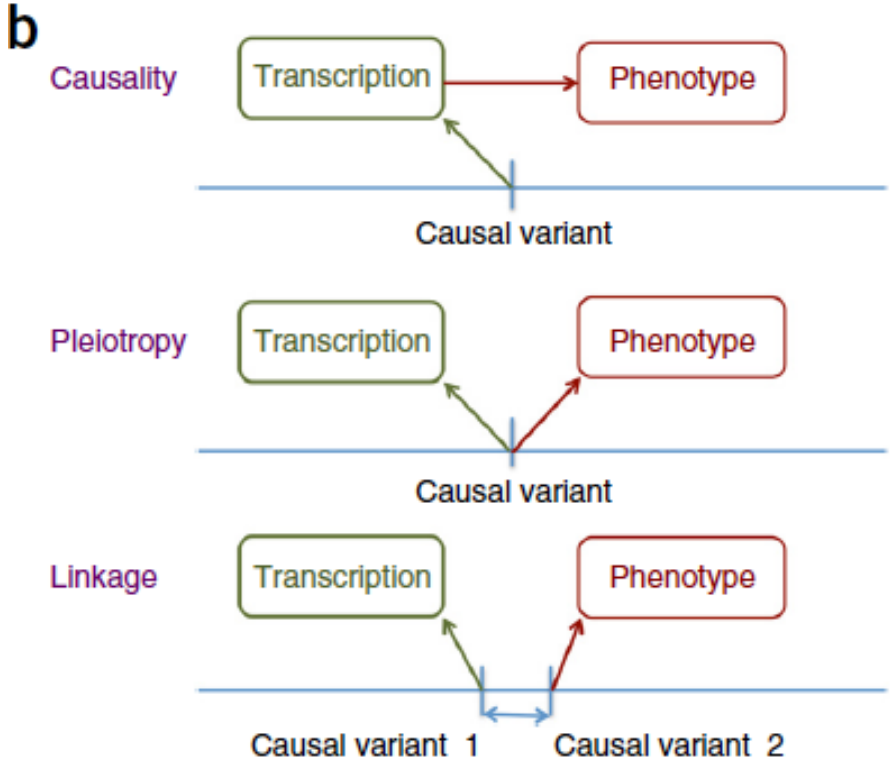
code is provided to apply these weights in practice.

Pleiotropy and sources of heterogeneity

Pleiotropy: same causal variant influencing exposure and outcome

Linkage: 2 variants in LD each influencing exposure and outcome

Other such as partial pleiotropy (direct SNP effect on a subset of phenotype)



Bowden et al.; Improving the accuracy of two-sample summary data Mendelian randomization: moving beyond the NOME assumption

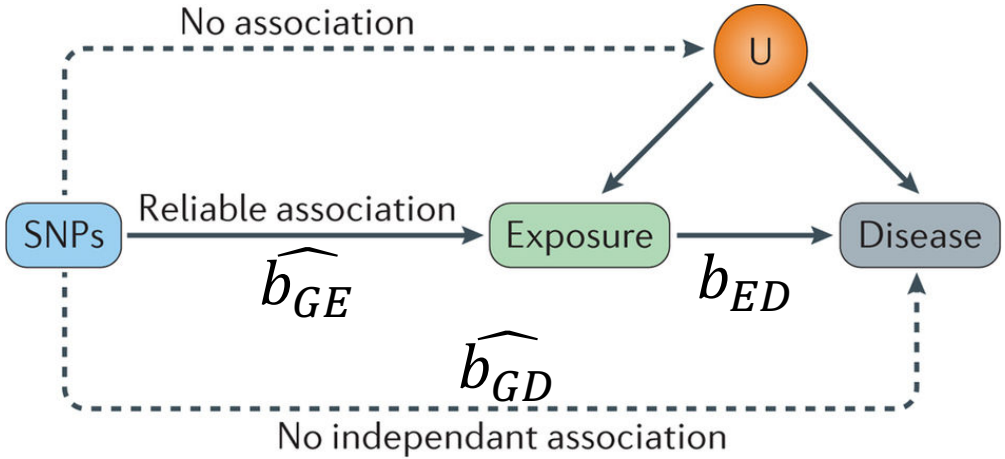
Zhu et al., Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets

Pleiotropy and heterogeneity tests

Under MR assumptions (SNP j)

$\widehat{b_{ED(j)}} = \widehat{b_{GD(j)}} / \widehat{b_{GE(j)}}$

Using $\widehat{b_{GD(j)}}$ and $\widehat{b_{GE(j)}}$ estimated from GWAS



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If SNP i effect is pleiotropic or linkage

$\widehat{b_{ED(i)}} \neq \widehat{b_{GD(i)}} / \widehat{b_{GE(i)}}$

Possible bias in MR effect size in presence of pleiotropy

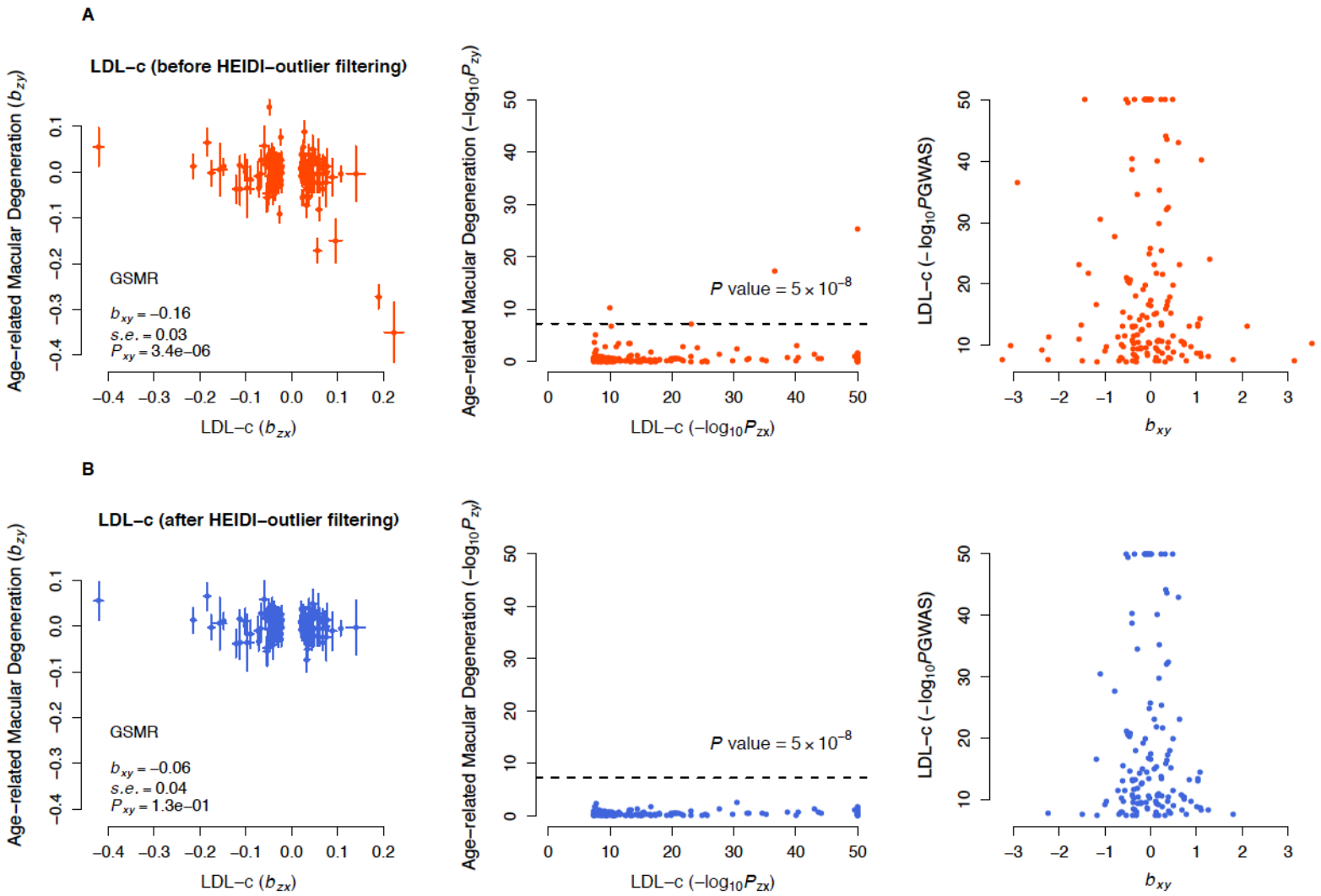
Heterogeneity tests

Method	Ref.	Test(s)
HEIDI	Zhu et al,. 2018 (Nat. com.)	Outlier test for instruments Covariate effect (mtCOJO)
Q modified	Bowden et al., 2017 (biorXiv)	Global test of heterogeneity (Cochran's Q modified – IVW framework)
Q' modified	Bowden et al., 2017 (biorXiv)	Global test of heterogeneity (Rucker's Q' modified – MR-EGGER framework)
MR-PRESSO	Verbank et al., 2018 (Nat. Gen.)	Global test of heterogeneity Outlier test for instruments Covariate adjustment

Global vs. outliers test

Outliers test assume a mixture of causality and pleiotropy => the causal effect may be estimated after removing SNPs pleiotropic or in linkage

Global tests may be more conservative and reject causal associations in presence of any heterogeneity
They may also be used to test residual heterogeneity after outlier exclusion



Zhu et al., GSMR paper

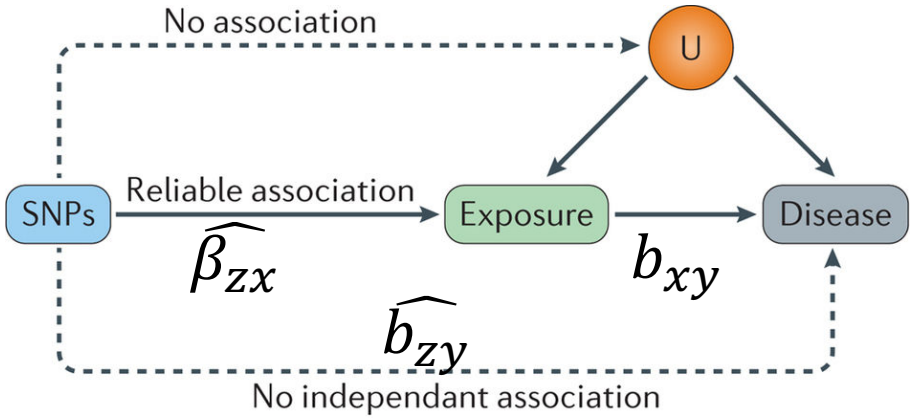
HEIDI (HEterogeneity In Dependent Instrument) test

$$b_{xy(i)} = \frac{b_{zy(i)}}{\beta_{zx(i)}}$$

$$d_i = b_{xy(i)} - b_{xy(\text{top})}$$

$$z_{d(i)} = \hat{d}_i \Big/ \sqrt{\text{var}(\hat{d}_i)}$$

Top is the most associated variant in 3/5th quintile



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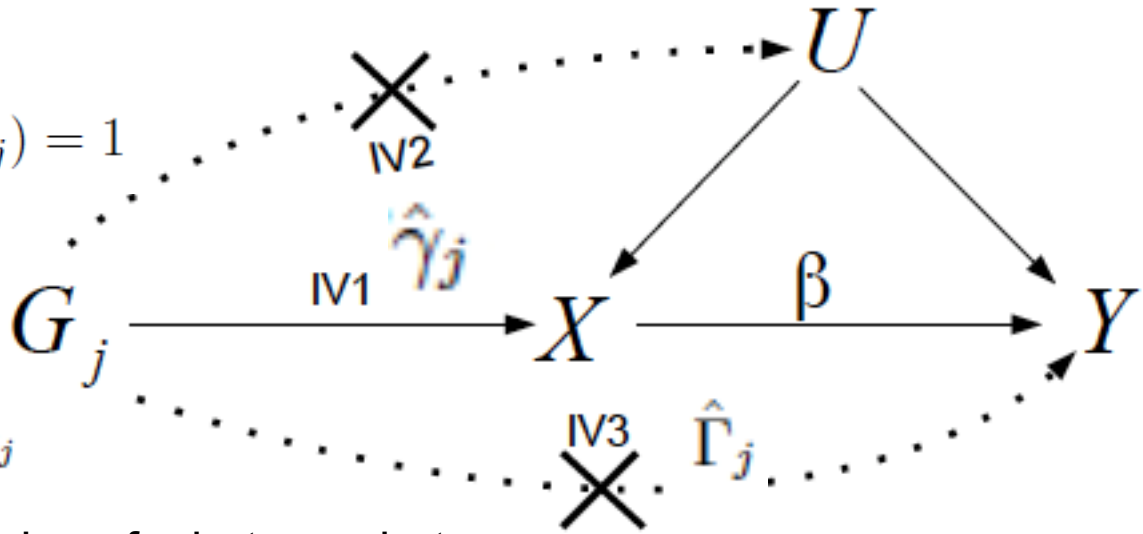
$$\mathbf{z}_d \sim \text{MVN}(\mathbf{0}, \mathbf{R})$$ Under H0 (causality)

$$T = \hat{d}_i^2 / \text{var}(\hat{d}_i)$$ Test performed for each instrument i, T~ $\chi(1)$

Q, Q' & MR-PRESSO: notation framework

$$\hat{\Gamma}_j = \beta\gamma_j + \sigma_{Yj}\epsilon_j, \quad \text{var}(\epsilon_j) = 1$$

True model



$$\hat{\Gamma}_j = \beta\hat{\gamma}_j + \sqrt{\beta^2\sigma_{Xj}^2 + \sigma_{Yj}^2}\epsilon_j$$

Fitted model: linear regression of **Γ**-hat on γ-hat

σ_{Yj}^2 represents the variance of $\hat{\Gamma}_j^2$ and σ_{Xj}^2 represents the variance of $\hat{\gamma}_j^2$.

$$\hat{\beta}_j = \beta + \sqrt{\frac{\hat{\Gamma}_j^2}{\hat{\gamma}_j^4}\sigma_{Xj}^2 + \frac{\sigma_{Yj}^2}{\hat{\gamma}_j^2}}\epsilon_j.$$

Q tests – Inverse Variance Weighted framework

$$\hat{\beta}_{IVW} = \frac{\sum_{j=1}^{L} w_j \hat{\beta}_j}{\sum_{j=1}^{L} w_j} \quad \text{where} \quad w_j = var(\hat{\beta}_j)^{-1},$$

$$Q = \sum_{j=1}^{L} Q_j = \sum_{j=1}^{L} w_j (\hat{\beta}_j - \hat{\beta}_{IVW})^2.$$

Heterogeneity of MR estimates across instruments

follow, asymptotically, a χ^2 distribution on L-1 degrees of freedom

$$\text{1st order weights:} \quad w_j = \frac{\hat{\gamma}_j^2}{\sigma_{Yj}^2}$$

$$\text{2nd order weights:} \quad w_j = \left(\frac{\sigma_{Yj}^2}{\hat{\gamma}_j^2} + \frac{\hat{\Gamma}_j^2 \sigma_{Xj}^2}{\hat{\gamma}_j^4} \right)^{-1}$$

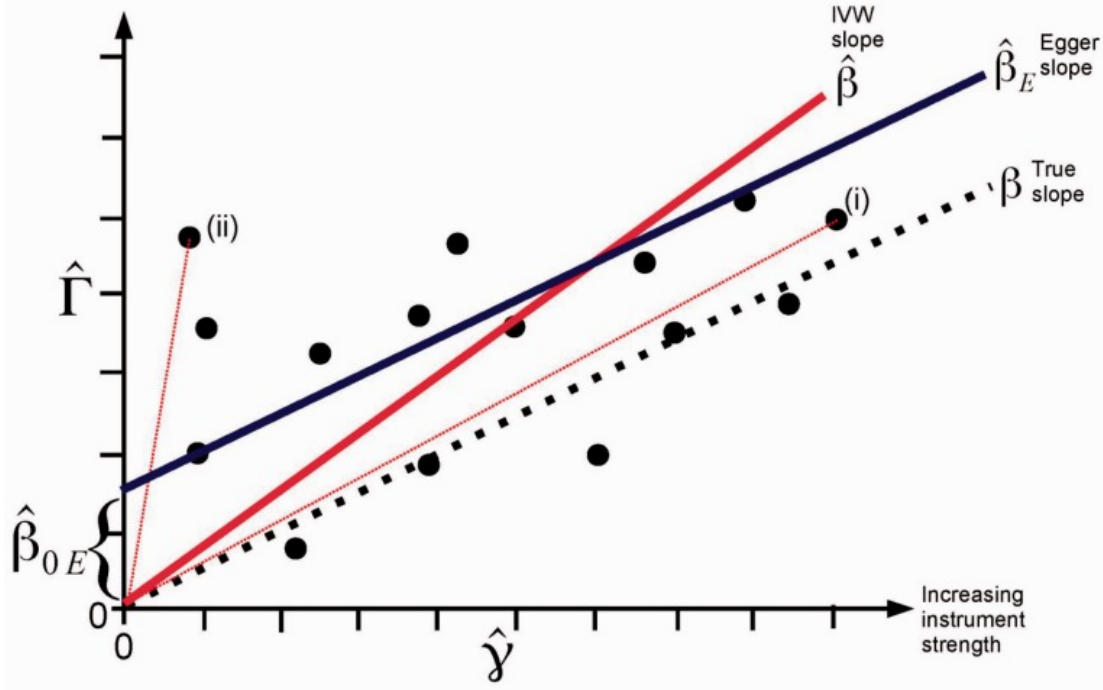
Improves Q test false positive rate and power

Bowden, et al., 2017; Improving the accuracy of two-sample summary data Mendelian randomization: moving beyond the NOME assumption

Q' tests – MR-EGGER

Linear regression of $\hat{\Gamma}_j$ on $\hat{\gamma}_j$ with intercept (beta0) and slope (beta1)

$$\hat{\beta}_j = \frac{\beta_{0E}}{\hat{\gamma}_j} + \beta_{1E} + w_j^{-\frac{1}{2}}\epsilon_j.$$



$$Q' = \sum_{j=1}^{L} Q'_j = \sum_{j=1}^{L} w_j(\hat{\beta}_j - \frac{\hat{\beta}_{0E}}{\hat{\gamma}_j} - \hat{\beta}_{1E})^2,$$

Similar as before with an intercept that may capture small study bias, follows a χ^2_{L-2} distribution

***Bowden et al., 2015**; Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression; **Bowden, et al., 2017**; Improving the accuracy of two-sample summary data Mendelian randomization: moving beyond the NOME assumption*

MR-PRESSO (global test)

MR-PRESSO global test. The MR-PRESSO global test evaluates for the presence of horizontal pleiotropy and is made up of four steps (Fig. 1a):

(1) For each variant j, we removed the variant in question and refit a IVW regression. This allowed us to calculate the slope of the regression line on the remaining variants, denoted $\hat{\beta}_{-j}$, which represents the causal estimate without variant j.

(2) The estimated causal effect (slope) without variant j was used to predict the expected effect size on the outcome as the product of $\hat{\beta}_{-j}$ and the effect size of the same variant on the exposure $\hat{\gamma}_j$. Then we calculated the observed residual sum of squares (RSS) as the difference between the observed effect size of the variant on the outcome ($\hat{\Gamma}_j$) and the predicted effect size of the same variant on the outcome $\mathrm{RSS}_{\mathrm{obs}}(j) = (\hat{\Gamma}_j - \hat{\beta}_{-j}\hat{\gamma}_j)^2$. The global observed RSS was then obtained by summing over the J $\mathrm{RSS}_{\mathrm{obs}}(j)$:

$$\mathrm{RSS}_{\mathrm{obs}} = \sum_j \mathrm{RSS}_{\mathrm{obs}}(j) = \sum_j (\hat{\Gamma}_j - \hat{\beta}_{-j}\hat{\gamma}_j)^2$$

Heterogeneity # sum of squares of betaj - beta-j

MR-PRESSO (global test - distribution)

(3) The observed RSS was compared with a simulated expected distribution of RSSs. The expected distribution was simulated under the null hypothesis (0% of variants are outliers). First, we simulated a distribution of effect sizes on the exposure $\hat{\gamma}_j^{\text{random}}$ from a Gaussian distribution $\mathcal{N}(\hat{\gamma}_j, \mathbb{V}(\hat{\gamma}_j))$. Second, we simulated a distribution of effect sizes on the outcome $\hat{\Gamma}_j^{\text{random}}$ around the predicted effect size on the outcome $(\hat{\beta}_{-j}\hat{\gamma}_j)$ by drawing in a Gaussian distribution $\mathcal{N}(\hat{\beta}_{-j}\hat{\gamma}_j, \mathbb{V}(\hat{\Gamma}_j))$. The expected RSS is then obtained as

$$\sum_j \text{RSS}_{\text{exp}}(j) = (\hat{\Gamma}_j^{\text{random}} - \hat{\beta}_{-j}\hat{\gamma}_j^{\text{random}})^2$$

The procedure was repeated multiple times (K) to obtain a null distribution of the K expected RSSs,

$$\text{RSS}_{\text{exp}}^k = \sum_j \text{RSS}_{\text{exp}}^k(j) = \sum_j (\hat{\Gamma}_{jk}^{\text{random}} - \hat{\beta}_{-j}\hat{\gamma}_{jk}^{\text{random}})^2$$

MR-PRESSO (outlier test)

MR-PRESSO outlier test. The MR-PRESSO outlier test allows for the detection of specific horizontal pleiotropic outlier variants. For a given variant *j*, we compared the observed *j*th RSS $RSS_{obs}(j)$ (obtained in step 2 of the global test) with the distribution of *K* expected *j*th RSSs $RSS^{k}_{exp}(j)$ (obtained in step 3 of the global test). Finally, an empirical *P* value is computed as

$$P_j = \frac{\sum_k 1_{>RSS_{obs}(j)} (RSS^{k}_{exp}(j))}{K}$$

(Fig. 1b), which is then multiplied by the number of variants J to account for multiple testing using the Bonferroni correction.

Given that *J* outlier tests are performed, we recommend use of a Bonferroni correction of the *J P* values.

Heterogeneity tests - Summary

Method	Ref.	Test(s)	Distribution	Pros/cons
HEIDI	Zhu et al,. 2018 (Nat. com.)	$(b_j - b_{top})$ **2	Approx. $\chi(1)$	Assumes b_{top} unbiased Global heterogeneity test not implemented
Q modified	Bowden et al., 2017 (biorXiv)	$\Sigma(b_j - b)$ **2	Approx. $\chi(m-1)$	Assumes b_{IVW} unbiased Outlier test can be constructed
Q' modified	Bowden et al., 2017 (biorXiv)	$\Sigma(b_j - b + b0)$ **2	Approx. $\chi(m-2)$	Assumes $b_{MR-EGGER}$ unbiased Outlier test can be constructed
MR-PRESSO	Verbank et al., 2018 (Nat. Gen.)	$(b_j - b_{-j})$ **2 $\Sigma(b_j - b_{-j})$ **2	Empirical	Assumes b_{IVW-j} unbiased Longer computationally

HEIDI, Q and Q' use saddlepoint approximation for distributions of quadratic forms in normal variables

Kuonen, 1999; Saddlepoint approximations for distributions of quadratic forms in normal variables

Lynch & Walsh; 1998 ("Delta method")

MR-PRESSO vs. Q or Q’

Supplementary Table 1: False positive rate to detect horizontal pleiotropy in Mendelian randomization.

Main causal effect	Global MR-PRESSO	Q test	Q (modified) test	Q’ test	Q' (modified) test
0	5.39	5.46	5.46	4.52	4.5
0.1	4.73	5.33	4.77	4.34	3.85
0.2	5.37	7.83	5.52	6.5	4.45
0.5	5.06	24.92	4.93	21.37	4.31

False positive rate (%; at a significance level of $P < 0.05$). A total of 50 variants was simulated in each case, with no horizontal pleiotropy. 10,000 replicates were simulated per scenario. MR-PRESSO: Mendelian Randomization Pleiotropy RESidual Sum and Outlier.

Comparable FPR and power

Table 1 | Power to detect horizontal pleiotropy in MR, for different methods

Causal effect	Horizontal pleiotropic variants (%)	Type of pleiotropy	MR-PRESSO global test	Q (modified) test	Q' (modified) test
0	2	Balanced	25.34	25.40	22.04
0	2	Positive	25.01	25.01	22.00
0	4	Balanced	51.79	51.96	47.80
0	4	Positive	50.88	51.32	47.34
0	10	Balanced	95.53	95.58	94.47
0	10	Positive	94.27	94.26	92.85
0.1	2	Balanced	24.10	24.41	21.58
0.1	2	Positive	23.60	23.89	20.96
0.1	4	Balanced	51.17	51.49	47.51
0.1	4	Positive	50.67	50.59	46.69
0.1	10	Balanced	95.56	95.56	94.31
0.1	10	Positive	93.73	93.77	92.08
0.2	2	Balanced	22.42	22.70	19.74
0.2	2	Positive	22.95	22.92	19.97
0.2	4	Balanced	48.37	48.29	44.45
0.2	4	Positive	46.89	46.82	43.18
0.2	10	Balanced	94.08	94.11	92.78
0.2	10	Positive	91.85	91.91	90.24
0.5	2	Balanced	16.72	16.70	14.90
0.5	2	Positive	16.76	16.55	15.15
0.5	4	Balanced	33.71	33.79	31.00
0.5	4	Positive	32.52	32.66	29.93
0.5	10	Balanced	81.15	81.22	79.07
0.5	10	Positive	76.99	77.09	74.75

The simulation scenarios included variations of the causal effect of exposure 1 on the outcome, the percentage of horizontal pleiotropic variants among the total of 50 variants and the type of pleiotropy. The InSIDE condition was satisfied in all reported scenarios.

HEIDI global test

$T_{heidi} = \Sigma(b_j - b_{top})^{**}2$ would be the direct extension of the HEIDI outlier test
$T_{heidi} \sim \chi(m-1)$ (Approx. for independent SNPs)

Still dependent on the choice of top instrument
Can we derive a test that uses all pair-wise contrasts?

$T_{heidi\text{-}full} = Z_d \, A \, Z_d'$

With Z_d is a matrix with $Z_d(i,j) = (b_j - b_i)/ sd(b_j - b_i)$
With A a matrix of variance covariance of Z_d , which may be calculated from SNP LD, and summary statistics (see SMR paper)

$T_{heidi\text{-}full}$ is a quadratic form, whose distribution may be approximated as a mixture of chi-squares with non-centrality parameters

HEIDI global test

1. Introduction

Let $X = (X_1, \ldots, X_n)^{\mathrm{T}}$ be a multivariate normal random vector with mean vector $\mu = (\mu_1, \ldots, \mu_n)^{\mathrm{T}}$ and covariance matrix Ω. The quadratic form associated with the $n \times n$ matrix A is defined as

$$Q(X) = X^{\mathrm{T}} A X = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} X_i X_j; \tag{1}$$

without loss of generality we assume that A is symmetric. Quadratic forms enter into many statistics associated with normally distributed random variables, so we may want to calculate

$$\mathrm{pr}\{Q(X) > q\}, \tag{2}$$

where q is a given scalar. In the simplest case, $A = \Omega = I_n$ and $Q(X)$ is a noncentral chi-squared variable with n degrees of freedom and noncentrality parameter $\xi^2 = \sum \mu_i^2$ (Scheffé, 1959, Appendix IV). If the matrix A is neither idempotent nor positive definite, classical results such as Cochran's theorem (Scheffé, 1959, Appendix VI) implying a chi-squared distribution for the quadratic form do not apply, and another approach to the calculation of (2) is needed. Johnson & Kotz (1970, Ch. 29) discuss representations of the distributions of quadratic forms. Imhof (1961) gives exact methods for computing (2) using real arithmetic. This method has been programmed in Fortran

Kuoken, 1999; Saddlepoint Approximations for Distributions of Quadratic Forms in Normal Variables

Something else

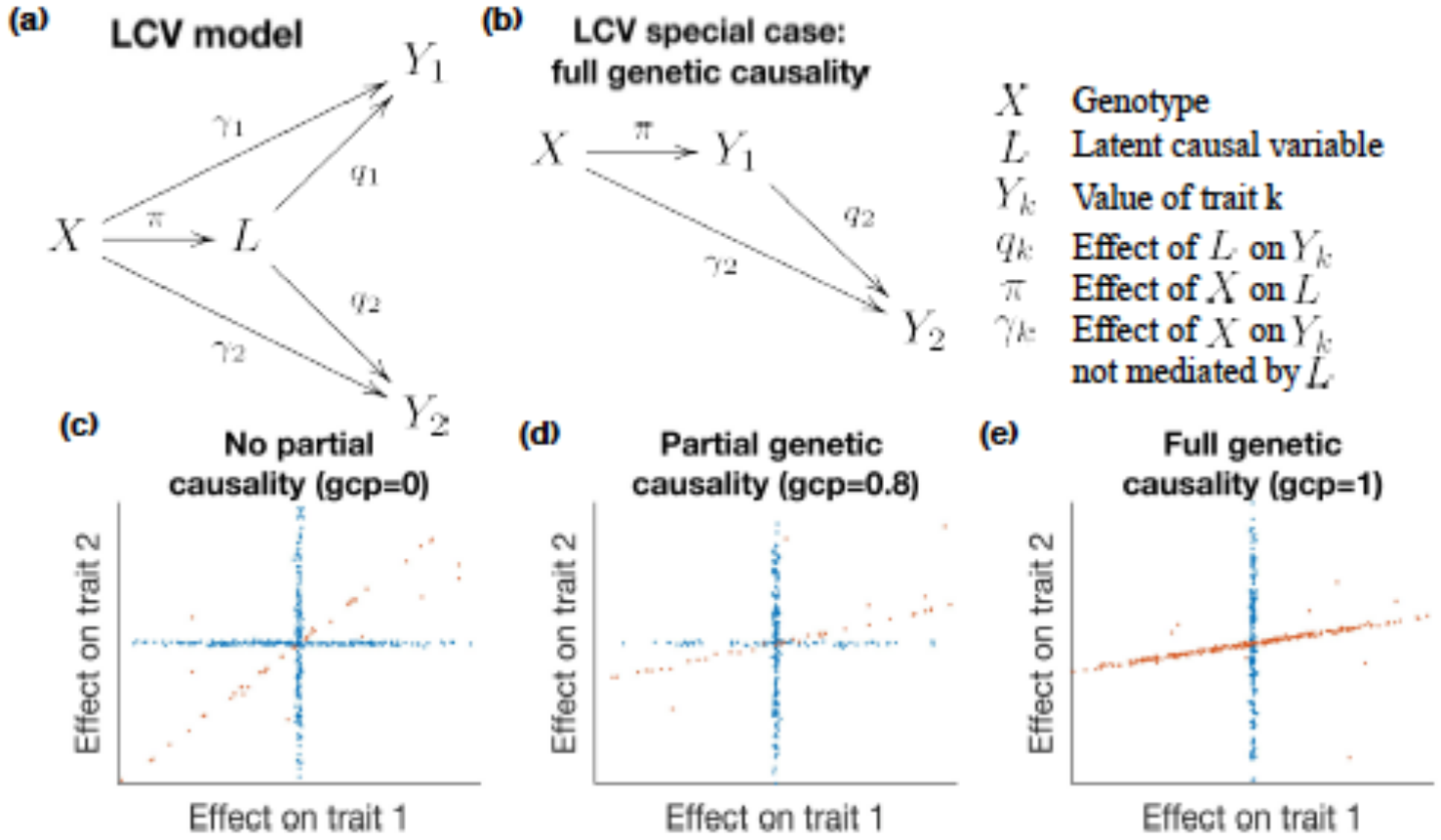
Distinguishing genetic correlation from causation across 52 diseases and complex traits

Luke J. O'Connor[1,2] and Alkes L. Price[1,3,4]

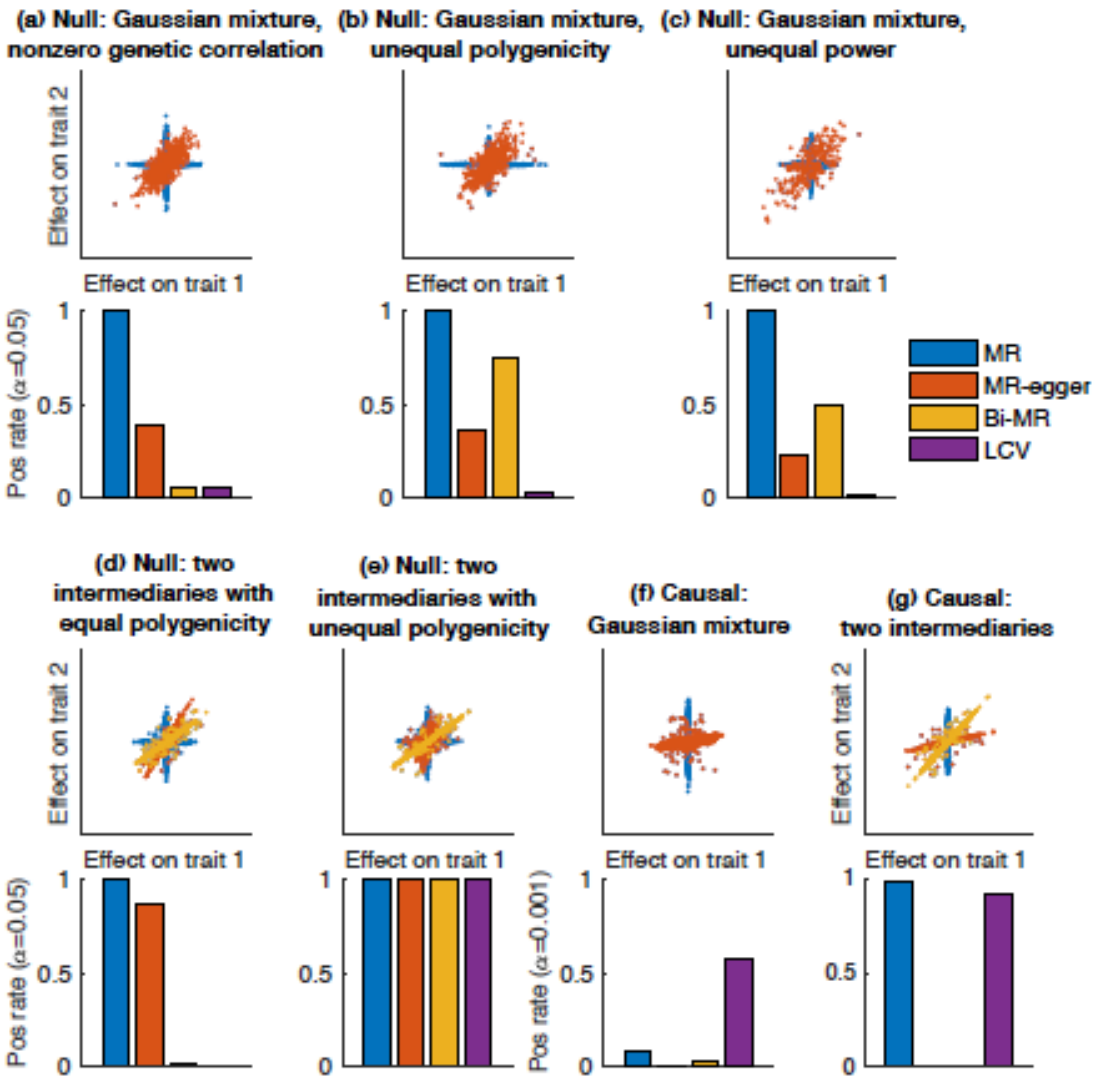
Abstract

Mendelian randomization (MR) is widely used to identify causal relationships among heritable traits, but it can be confounded by genetic correlations reflecting shared etiology. We propose a model in which a latent causal variable mediates the genetic correlation between two traits. Under the latent causal variable (LCV) model, trait 1 is *fully genetically causal* for trait 2 if it is perfectly genetically correlated with the latent causal variable, implying that the entire genetic component of trait 1 is causal for trait 2; it is *partially genetically causal* for trait 2 if it has a high genetic correlation with the latent variable, implying that part of the genetic component of trait 1 is causal for trait 2. To quantify the degree of partial genetic causality, we define the *genetic causality proportion* (gcp). We fit this model using mixed fourth moments $E(\alpha_1^2\alpha_1\alpha_2)$ and $E(\alpha_2^2\alpha_1\alpha_2)$ of marginal effect sizes for each trait, exploiting the fact that if trait 1 is causal for trait 2 then SNPs affecting trait 1 (large α_1^2) will have correlated effects on trait 2 (large $\alpha_1\alpha_2$), but not vice versa. We performed simulations under a wide range of genetic architectures and determined that LCV, unlike state-of-the-art MR methods, produced well-calibrated false positive rates and reliable gcp estimates in the presence of genetic correlations and asymmetric genetic architectures; we also determined that LCV is well-powered to detect a causal effect. We applied LCV to GWAS summary statistics for 52 traits (average N=331k), identifying partially or fully genetically causal effects (1% FDR) for 59 pairs of traits, including 30 pairs of traits with high gcp estimates ($\hat{\text{gcp}} > 0.6$). Results consistent with the published literature included genetically causal effects on myocardial infarction (MI) for LDL, triglycerides and BMI. Novel findings included a genetically causal effect of LDL on bone mineral density, consistent with clinical trials of statins in osteoporosis. These results demonstrate that it is possible to distinguish between genetic correlation and causation using genetic data.

Model


(a)
LCV model
Y_1
γ_1
q_1
X
π
L
q_2
γ_2
Y_2
(b)
LCV special case:
full genetic causality
X
π
Y_1
q_2
γ_2
Y_2
X Genotype
L Latent causal variable
Y_k Value of trait k
q_k Effect of L on Y_k
π Effect of X on L
γ_k Effect of X on Y_k not mediated by L
(c)
No partial causality (gcp=0)
Effect on trait 2
Effect on trait 1
(d)
Partial genetic causality (gcp=0.8)
Effect on trait 2
Effect on trait 1
(e)
Full genetic causality (gcp=1)
Effect on trait 2
Effect on trait 1

FPR and power



The presence of heterogeneity does not necessarily invalidate an MR study. For example if the underlying cause of the heterogeneity is pleiotropy but, across all variants, the amount of pleiotropy is independent of instrument strength (the InSIDE assumption [9]), then a standard random effects meta-analysis will still yield reliable inferences [3, 9]. If the pleiotropy is instead 'directional' (it has a non-zero mean) then a random effects meta-analysis will be biased, but MR-Egger regression [9] can still yield reliable inferences. MR-Egger regression has been used extensively as a

Bowden et al., Improving the accuracy of two-sample summary data Mendelian randomization: moving beyond the NOME assumption

Thank you