孟德尔随机化

Mendelian Randomization

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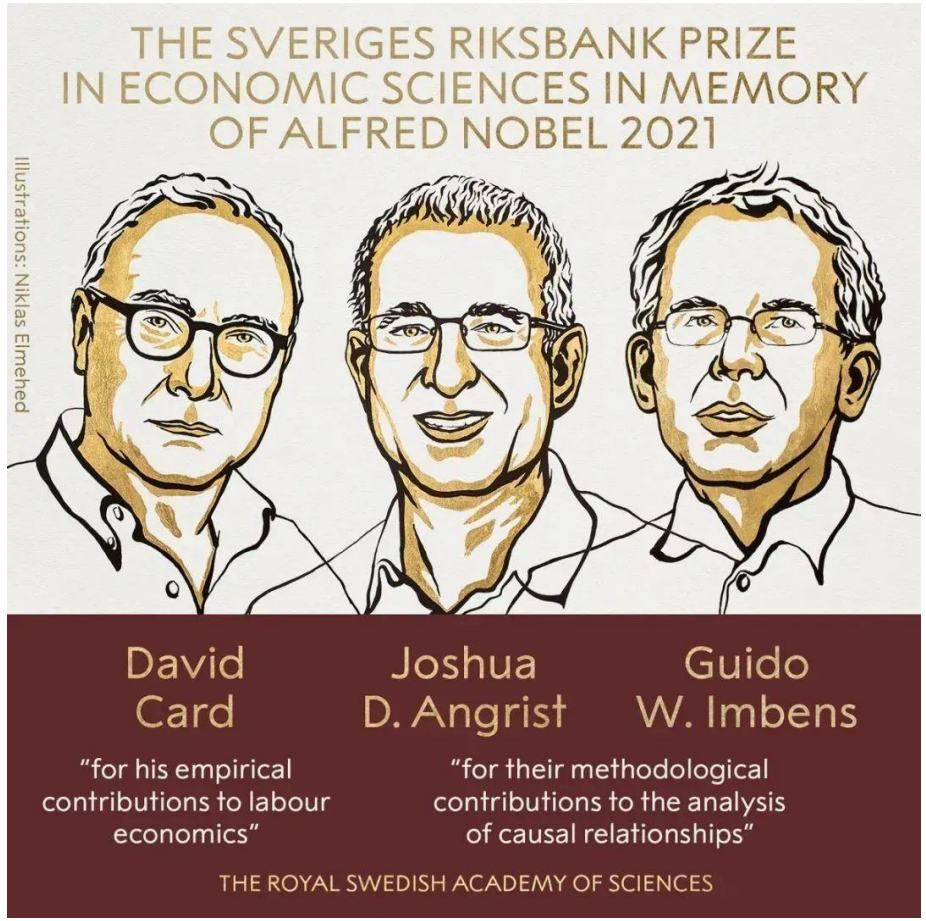
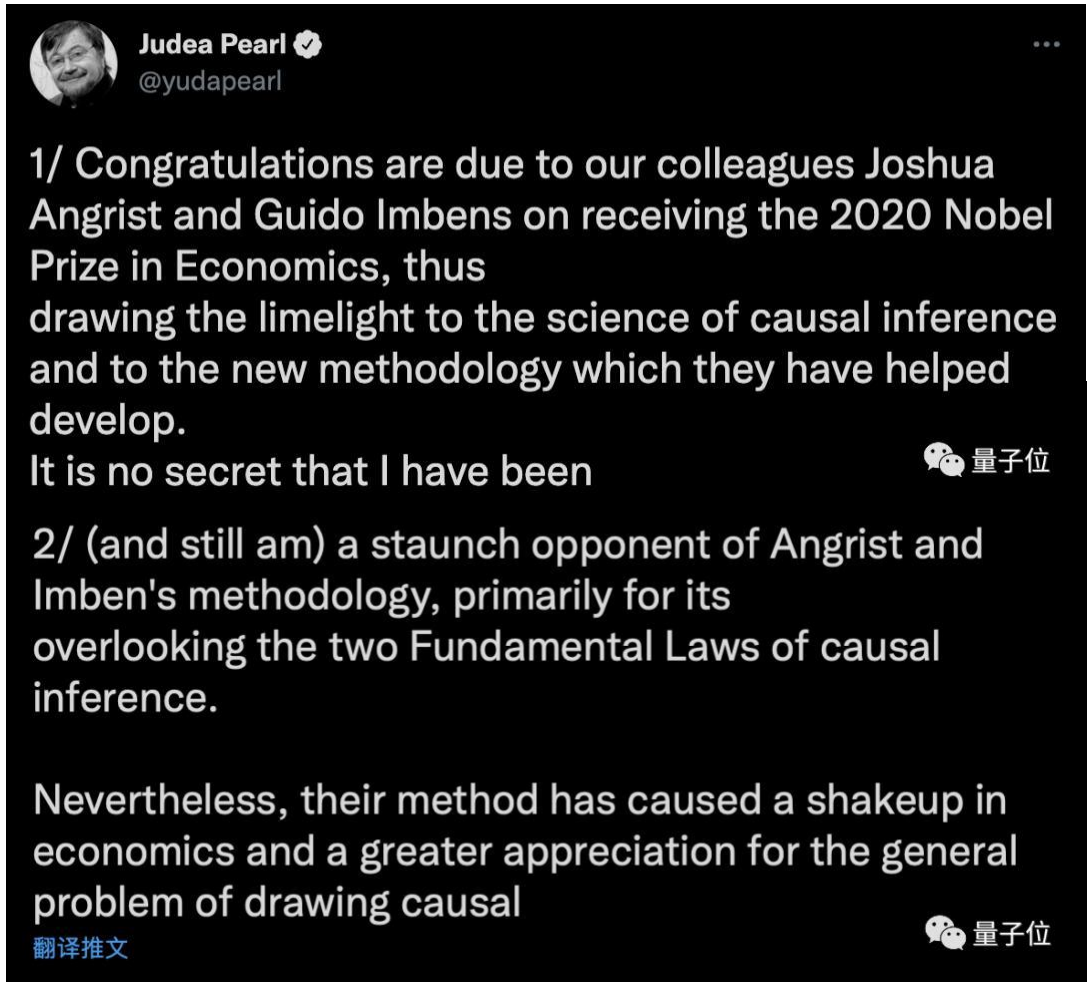
Causal inference



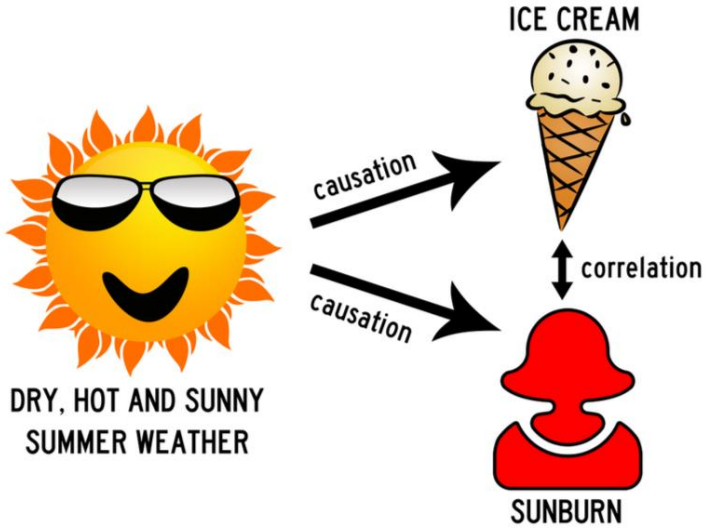
澎湃号 > 量子位QbitAI

因果推断研究获2021诺贝尔经济学奖，图灵奖得主Judea Pearl祝贺并反对



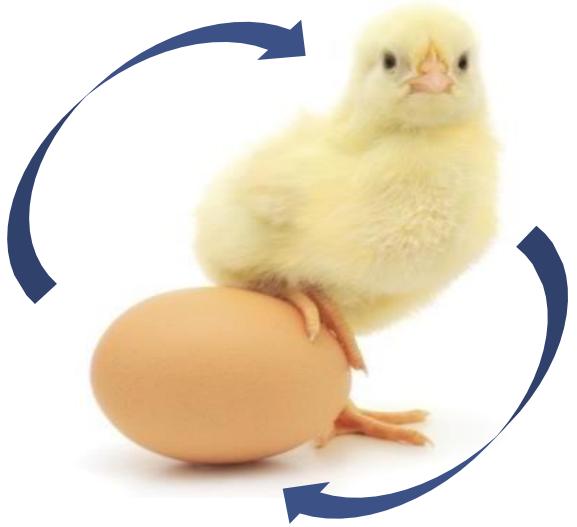
Causal inference

- Correlation ≠ causality



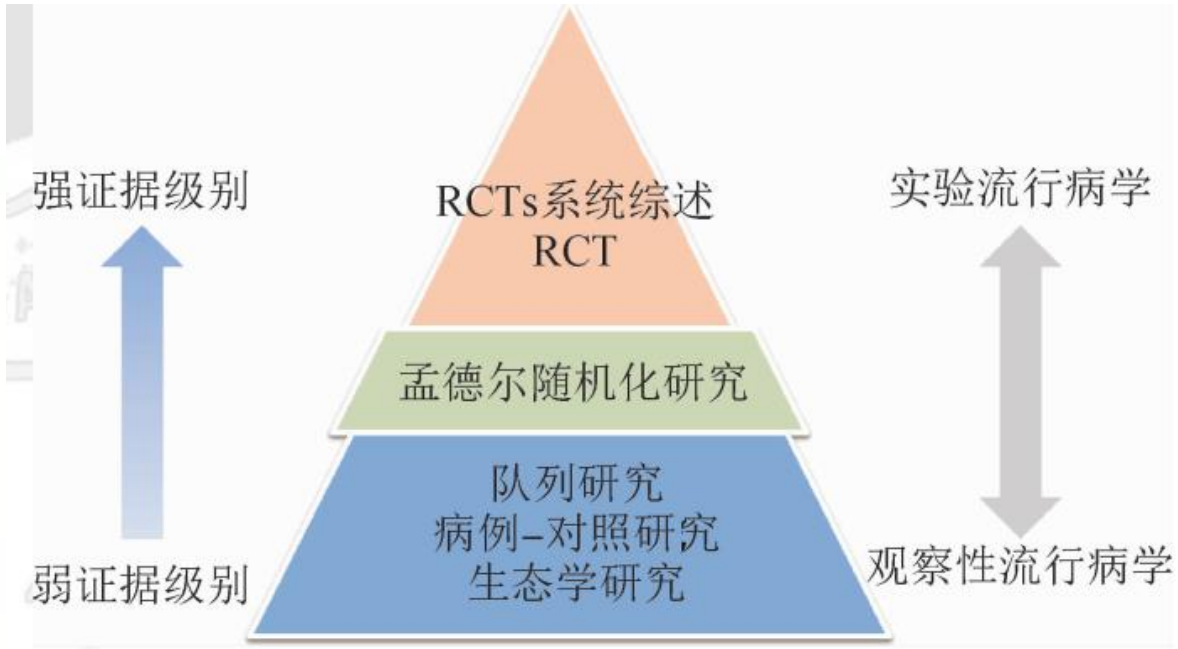
Confounding

Reverse causality



Bias

Real-world evidence



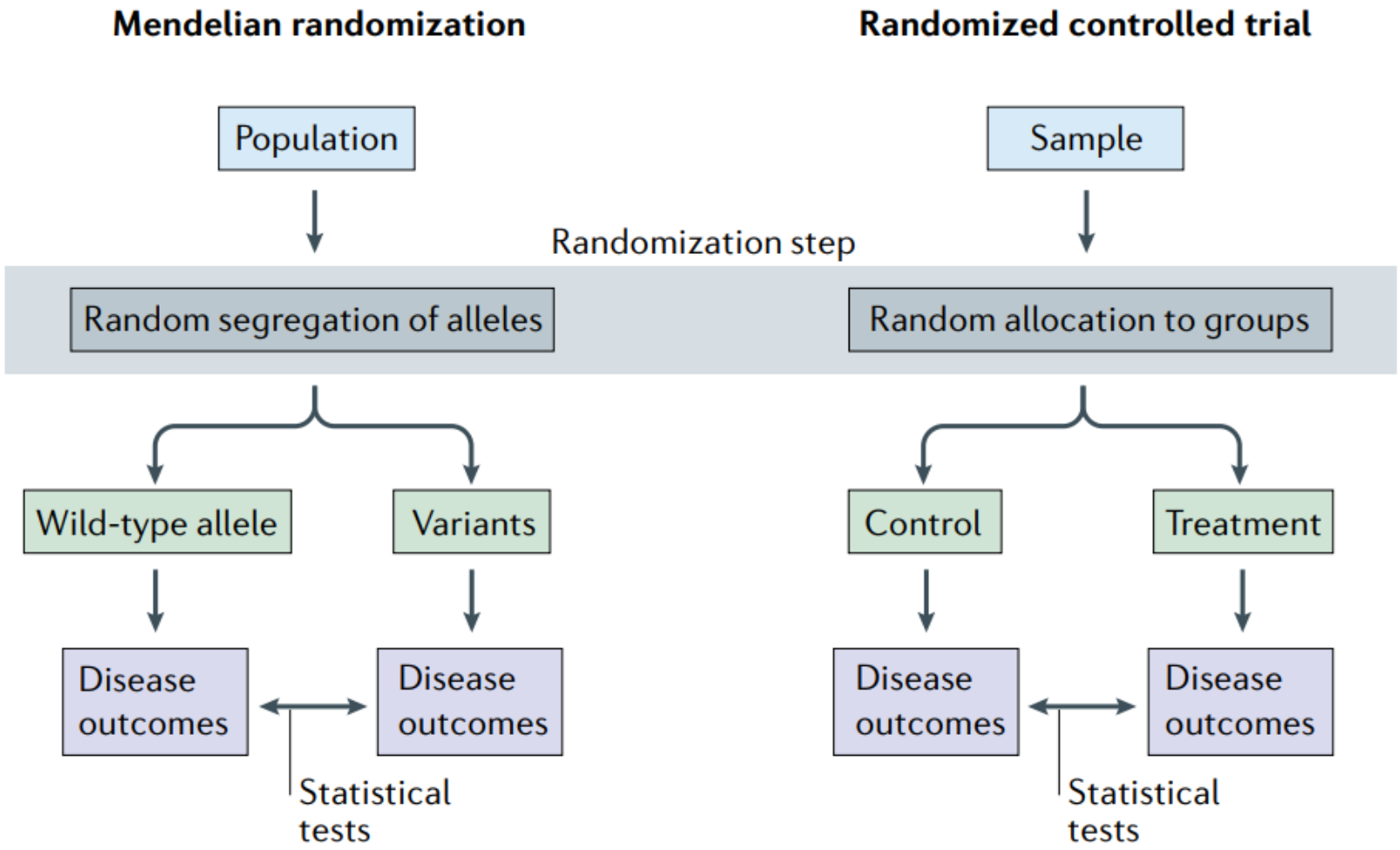
准实验研究

Outline

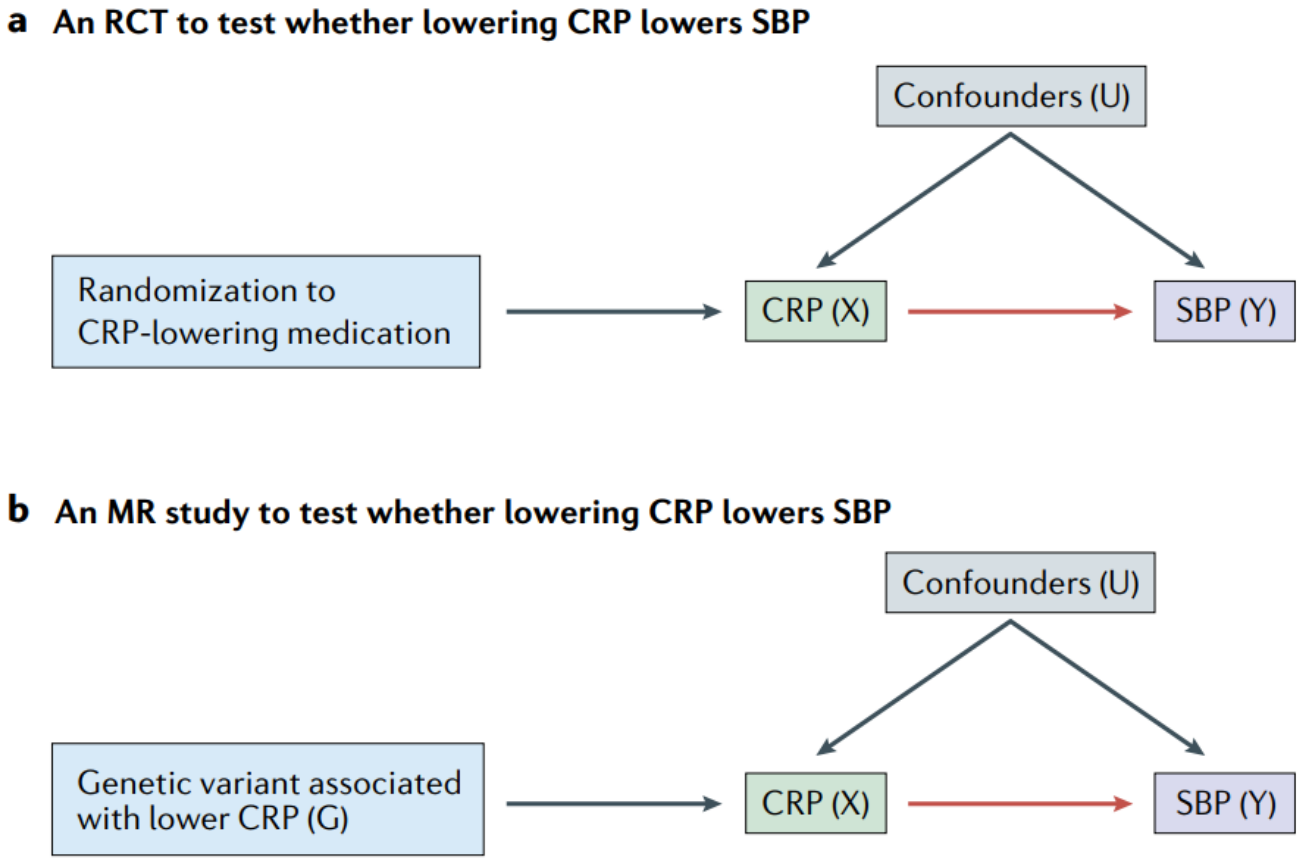
- MR的基本概念和假设
- 单样本和两样本MR估计方法
- MR设计的扩展
- 违背MR基本假设的情况及处理
- MR研究的局限性
- 如何开展MR研究

MR的基本概念和假设

From RCT to MR



RCT & MR



根据孟德尔遗传定律，严格来讲，只有Family-based MR等同于RCT，而population-based会由于人群分层、群体婚配结构等原因可能引入新的混杂

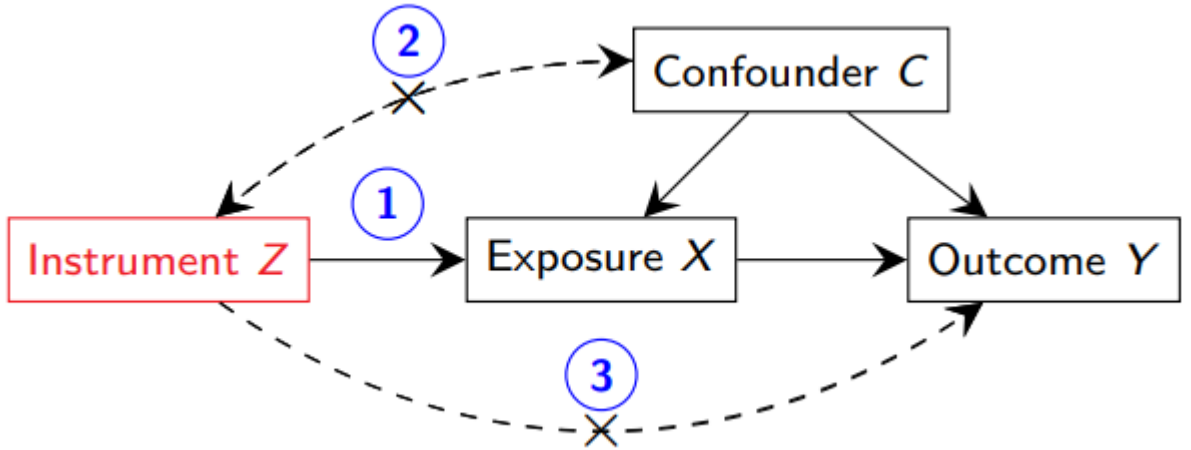
RCT & MR

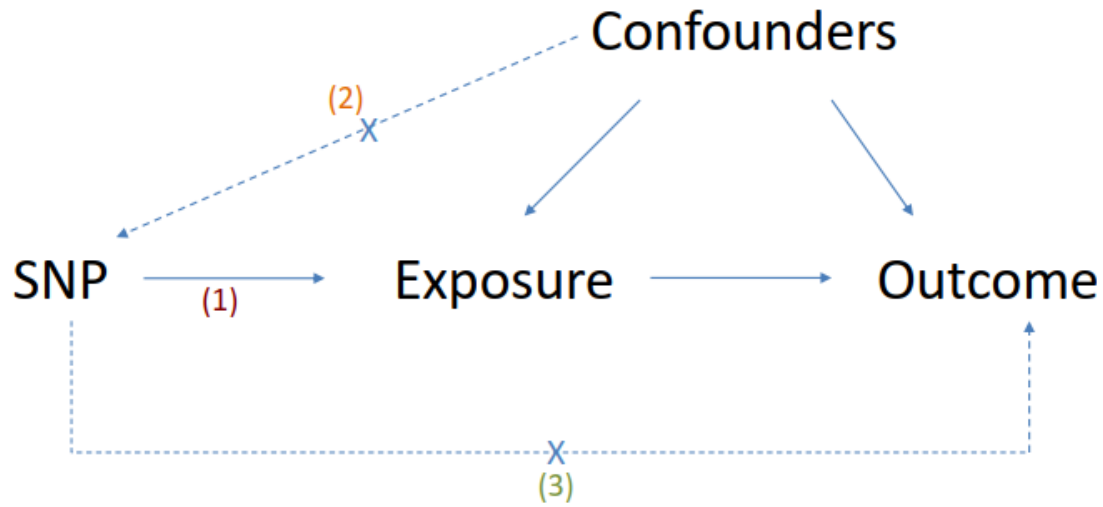
TABLE 1. Analogies between randomized controlled trials and Mendelian randomization studies

Randomized controlled trial	Mendelian randomization
Design and analysis	
Random allocation of treatment	Random allocation of genotype
Actual receipt of treatment	Intermediate phenotype or gene product that is influenced by the genotype
Disease outcome	Disease outcome
Intention-to-treat analysis: effect of random allocation on outcome in whole study population	Genotypic effect on outcome
"True" treatment effects: effect on outcome for persons who actually received the treatment	Effect of the intermediate phenotype or gene product on outcome
Possible sources of bias	
Unblinding: based on knowledge of the allocated treatment, doctors and/or patients adapt management/behavior during the course of the trial	Canalization: developmental adaptation to the genetically determined problem—"buffering"
Differential follow-up between randomized groups	Selective survival
Subgroup effects	Gene-environment interaction, gene-gene interaction

MR from an IV perspective

1. **Relevance**: *Z* is associated with the exposure (*X*).
2. **Exchangeability**: *Z* is independent of the unmeasured confounder (*C*).
3. **Exclusion restriction**: *Z* cannot have any direct effect on the outcome (*Y*).

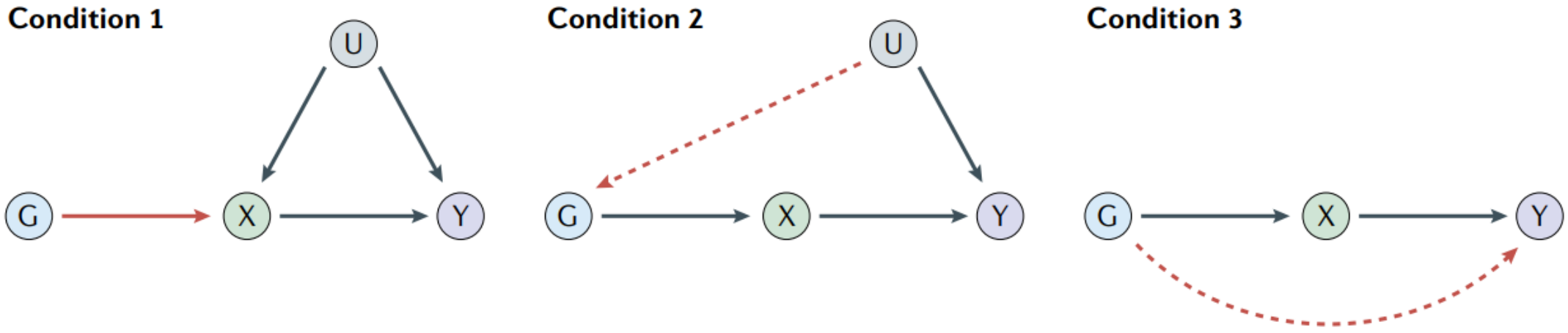




IV assumptions

- IV condition 1: relevance. The IV is associated with the exposure.
- IV condition 2: exchangeability. There are no causes of the IV that also influence the outcome through mechanisms other than the exposure of interest (no confounders of the IV and the outcome).
- IV condition 3: the exclusion restriction. The IV does not affect the outcome other than through the exposure and does not affect any other trait that has a downstream effect on the outcome of interest.

Only the first condition can be formally tested. The other two conditions can be disproved and otherwise assessed through a range of sensitivity analyses, but cannot be demonstrated to be true[66,209]. Methods for testing the first condition and of assessing the plausibility of the second and third conditions are discussed in the 'Results' section.

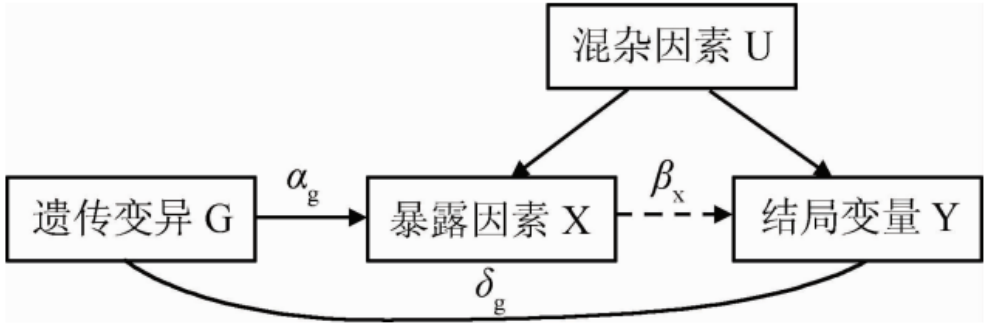


The 4th IV assumption: Point estimation

- 为了得到因果作用的点估计，事实上还需要第四个IV假设：
  1. homogeneity of the effect of the exposure on the outcome：
     - either (a) the effect of the exposure on the outcome is the same for everyone, regardless of the starting value of X or any other individual characteristics, or (b) the effect of the exposure on the outcome does not depend on the value of the instrument.
     - ACE
  2. monotonicity in the association between the genetic variants and the exposure
     - LATE

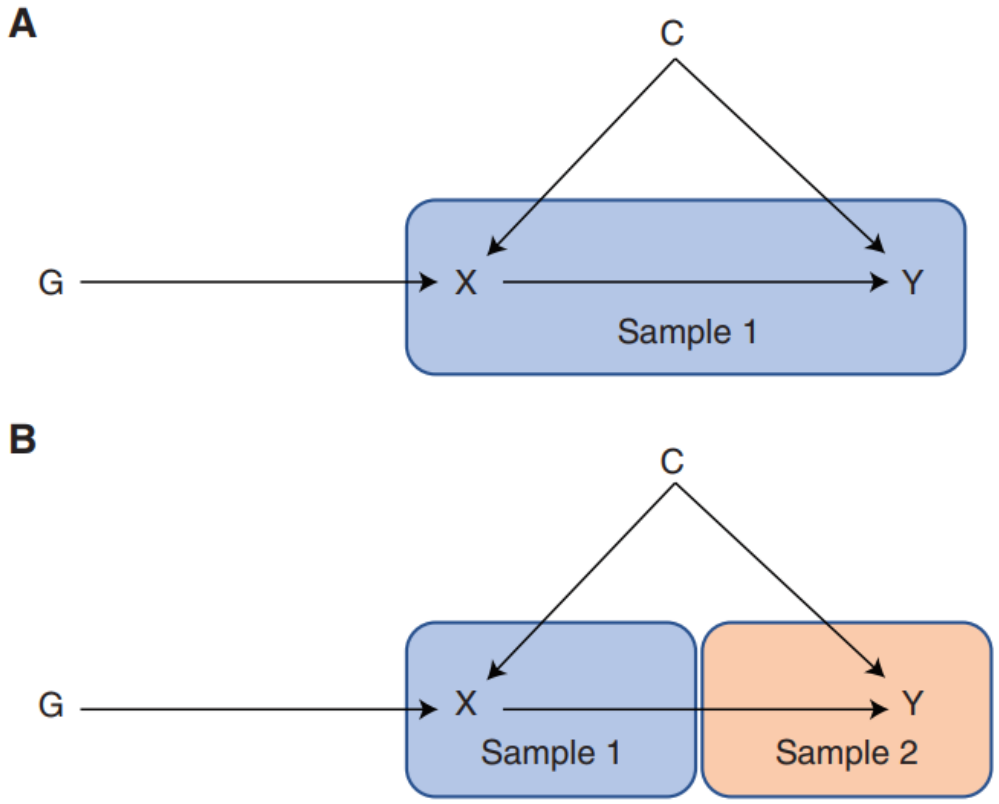
单样本和两样本MR估计方法

两种MR类型



- One-sample MR: individual-level data
  - G, X, Y来自同一样本的数据
  - 通常有个体水平数据

- two-sample MR: summary data
  - G-X和G-Y来自不同样本
  - 通常只有GWAS摘要统计量

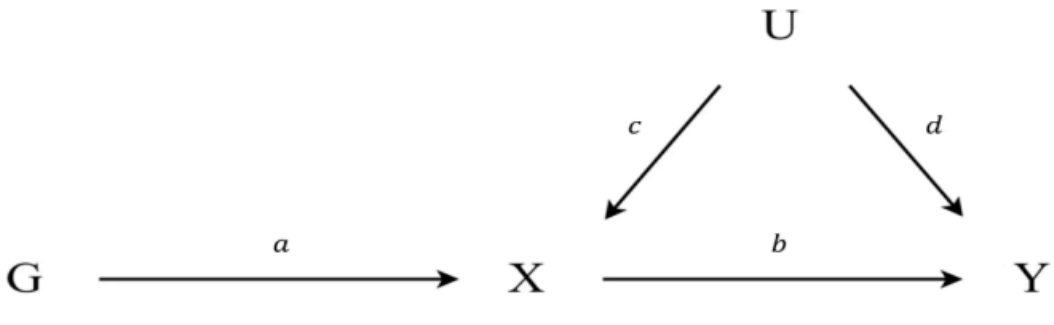


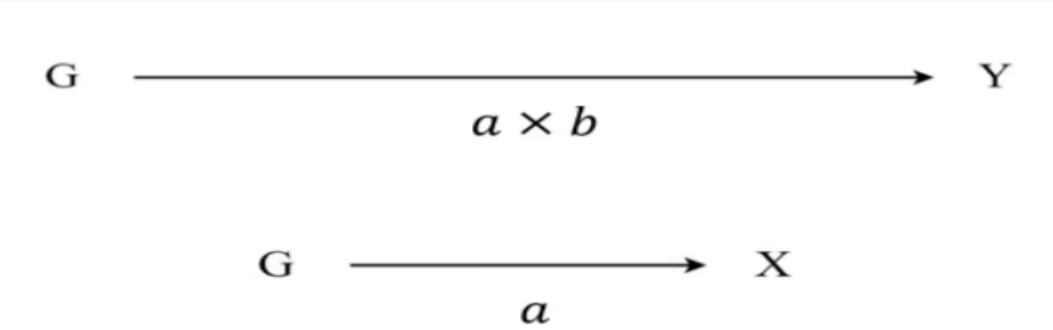
Wald ratio estimator

- 适用于只有一个IV时

$$\gamma_1 = \frac{E[\mathrm{SBP}|G=1] - E[\mathrm{SBP}|\mathrm{G}=0]}{E[\mathrm{CRP}|G=1] - E[\mathrm{CRP}|G=0]}$$

$$关联_{ZY} = 关联_{ZX} \times 关联_{XY},$$





Ratio Estimate

$$\frac{G-Y\ association}{G-X\ association} = \frac{a \times b}{a} = b$$

TSLS/2SLS

- 两阶段最小二乘法
  - 第一阶段：X~Z回归

$$X_i = \gamma_0 + \sum_j \gamma_j Z_{ij} + \varepsilon_{xi}$$

  - 第二阶段：Y~X第一阶段回归预测值回归

$$Y_i = \beta_0 + \beta_{\mathrm{IV}_j} \widehat{X}_{ij} + \varepsilon_{yi}$$

  - 多个IV：
    1. 全部加到回归项中
    2. 计算多基因风险评分PRS，把PRS作为IV

TSLS

- 矩阵表达：IV数与exposure数相同——即比值法（恰好识别）

When G and X are of same dimension (n>1), we have:

$$Y = X\beta + \varepsilon$$
$$X^T Y = X^T X\beta + X^T \varepsilon$$
$$G^T Y = G^T X\beta + G^T \varepsilon$$
$$\hat{\beta}_{IV} = (G^T X)^{-1}(G^T Y)$$
$$\sqrt{n}(\hat{\beta}_{IV} - \beta) \xrightarrow{p} N(0, \sigma^2 Q_{GX}^{-1} Q_{GG} Q_{XG}^{-1})$$

where $(G^T X/n) \xrightarrow{p} Q_{GX}, (G^T G/n) \xrightarrow{p} Q_{GG}$

TSLS

- 过度识别

When G and X are of different dimension (d(G)>d(X)), we have:

Two-stage least squares (2SLS) estimator:

$$\hat{\beta}_{IV} = (X^T G(G^T G)^{-1} G^T X)^{-1}(X^T G(G^T G)^{-1} G^T Y)$$

Asymptotic distribution:

$$\sqrt{n}(\hat{\beta}_{IV} - \beta) \xrightarrow{p} N(0, \sigma^2(Q_{GX} Q_{GG}^{-1} Q_{XG})^{-1})$$

Stage 1:

$$\hat{X} = X(G^T G)^{-1} G^T X$$

Stage 2:

$$\hat{\beta}_{IV} = (\hat{X}^T \hat{X})^{-1} \hat{X}^T Y$$

其他单样本MR估计方法

- 极大似然法：LIML
- Bayes估计
- 广义矩估计：GMM
- g-估计：SMM

$$\hat{\beta}_{\mathrm{IV}} = (\boldsymbol{Z}'\boldsymbol{X})^{-1}\boldsymbol{Z}'\boldsymbol{Y}$$

$$\begin{aligned}\hat{\beta}_{\mathrm{TSLS}} &= (\boldsymbol{X}'\boldsymbol{Z}(\boldsymbol{Z}'\boldsymbol{Z})^{-1}\boldsymbol{Z}'\boldsymbol{X})^{-1}(\boldsymbol{X}'\boldsymbol{Z}(\boldsymbol{Z}'\boldsymbol{Z})^{-1}\boldsymbol{Z}'\boldsymbol{Y}) \\ &= (\boldsymbol{X}'\boldsymbol{P}_{\boldsymbol{Z}}\boldsymbol{X})(\boldsymbol{X}'\boldsymbol{P}_{\boldsymbol{Z}}\boldsymbol{Y}) \\ &= (\boldsymbol{X}'\boldsymbol{P}_{\boldsymbol{Z}}'\boldsymbol{P}_{\boldsymbol{Z}}\boldsymbol{X})(\boldsymbol{X}'\boldsymbol{P}_{\boldsymbol{Z}}'\boldsymbol{P}_{\boldsymbol{Z}}\boldsymbol{Y}) \\ &= (\hat{\boldsymbol{X}}'\hat{\boldsymbol{X}})^{-1}\hat{\boldsymbol{X}}'\boldsymbol{Y}\end{aligned}$$

$$\beta_{\mathrm{GMM}} = (\boldsymbol{X}'\boldsymbol{Z}\boldsymbol{W}\boldsymbol{Z}'\boldsymbol{X})^{-1}(\boldsymbol{X}'\boldsymbol{Z}\boldsymbol{W}\boldsymbol{Z}'\boldsymbol{Y})$$

单样本IV方法

Method	Basic notion	Exposure effects	Strength	Limitation
Ratio estimator (RE)	-the RE is appropriate when only one IV	-RD, RR, OR	-simple estimation method -with a single binary IV and no other confounders, 2SLS = RE	-not suitable for multiple IVs -does not allow to adjust confounders - may not consistent for the causal OR
Two-stage least squares (2SLS)	-linear models without making parametric assumptions on the error terms -for multiple IVs, IV estimator is the weighted average of the ratio estimators	-estimator similar as classical regression	-natural starting point of IV analysis -the estimate asymptotically unbiased -widely used for binary exposure and outcome and provides the exposure effect on risk difference scale -unlike RE, it is able to adjust the possible measured confounders	-show biased results in binary cases or in the case of non-linear models -for multiple IVs, 2SLS estimator is biased and hence limited information of maximum likelihood method would be an alternative -for smaller sample sizes, limited information maximum likelihood estimator is more efficient and consistent than 2SLS -IV and 2SLS are a special case of GMM; however both yield the same results in the case of homoscedastic errors variance
Linear probability models (LPM)	-applied for binary outcome, exposure, and IV, the data are modelled using linear functions -for a single binary IV, the estimator equivalent to the RE	RD	-simple to estimate and interpret as the regression coefficients -the RD is consistent for the ACE	- sometimes predicted probabilities outside of the 0–1 range and for rare outcomes this may become negative - assumes the marginal/incremental effect of exposure remains constant which is logically impossible for binary outcome
Two-stage predictor substitution (2SPS)	-the rote extension to nonlinear models of the linear IV models -targets a marginal (population-averaged) odds ratio -it is the mimic of 2SLS -non-linear least squares is used to estimates the parameter -for a linear model, 2SPS = 2SLS	-RD, RR, OR	-suitable for non-linear association between exposure and outcome	-in practice, 2SPS in non-linear model does not always yield consistent exposure effects on the outcome - parameter estimation process is more difficult than 2SLS -under a logistic regression model, 2SPS may not provide causal OR
Two- stage residual inclusion (2SRI)	-include the estimated unobservable confounder (residual) from the first-stage as an additional variable along with the exposure in the second-stage model - also called control function estimator -under a linear model, 2SRI = 2SLS = 2SPS	-RD, RR, OR	-yields consistent estimates for linear and non-linear models -performs better than 2SPS -possible to apply in the specific case of a binary exposure with a binary or count outcome -for a log-linear model in the stage-two, 2SRI estimator provides CRR	-it may give biased estimates when there is strong unmeasured confounding, as is usually the case in an IV analysis -under a logistic regression model, 2SRI estimator may not provide causal OR -generally require the exposure to be continuous, rather than binary, discrete, or censored
Two-stage logistic regression (2SLR)	-when outcome and exposure are binary and interest to estimate OR -fully parametric, maximum likelihood technique is used to estimate the parameters	-OR	-parallel to 2SLS using LRM in both stages instead of linear models	-if the first-stage logistic model is not correctly specified then second-stage parameter estimates might be biased -estimator does not provide COR

单样本IV方法

Three-stage least squares (3SLS)	-an extension of 2SLS but unlike the 2SLS, all coefficients are estimated simultaneously, requires three steps -in 2SLS, if the errors in the two equations are correlated, the 3SLS can be an suitable alternative	-RD, RR	-more information is used and hence the estimators are likely to be more efficient than 2SLS	-more vulnerable to a misspecification of the error terms -very rarely applied in epidemiologic studies -estimation process is more complicated than 2SLS -3SLS becomes inconsistent if errors are heteroskedastic
Structural mean models (SMM)	-SMMs use IVs via G-estimation and involves the assumption of conditional mean independence -additive SMMs use continuous outcome and multiplicative SMMs use positive-valued outcomes -MSMM assumed log-linear model to measure the risk ratio -LSMM assumes logistic regression model which is fitted by maximum likelihood technique	RD, RR, OR	-it relaxes several of the modelling restrictions (constant treatment effects) required by ratio estimator/ two-stage methods -can be used in the case of time-dependent instruments, exposures, and confounders -provides average treatment effects for the treated subjects	-the assumption of no effect modification is impossible to verify -with a binary outcome, additive SMMs and MSMM suffer from the limitations of linear and log-linear models (e.g., predicted response probabilities may outside of the interval [0,]))
Generalized method of moments (GMM)	-a non-linear analogue of 2SLS -the standard IV (2SLS) estimator is a special case of a GMM estimator -making assumptions about the moments of the error term -allows estimation of parameters in over-identified model (number of IV greater than number of exposure variable) -the parameters are estimated in an iterative process	RD, RR, OR	-it requires specification only of certain moment conditions -applicable for the linear and non-linear models -non-linear GMM estimator is asymptotically more efficient than 2SLS -more robust and less sensitive to parametric conditions -works better than 2SLR when exposure and outcome are binary -in case of heteroskedasticity, this is more efficient than the linear IV estimators	-GMM estimator with logistic regression model is not consistent for the COR due to non-collapsibility of the OR
Bivariate probit models (BPM)	-two-stage method, but as different to 2SLS and model the probabilities directly and are restricted on [0,1] -full information maximum likelihood is used to estimate the parameter -accounts for the correlation between the errors	Probit coefficient*	-for binary outcome and exposure, BPM perform better than linear IV methods -the estimator of BPM have no interpretation like OR. However, by multiplying a probit coefficient by approximately 1.6, the estimator can be made to approximate OR	-when the distribution of error terms are not normal or the average probability of the outcome variable is close to one or zero, the BPM estimator may not be consistent for ACE

Two-sample methods

- 数据：G-X和G-Y的effect size及其估计标准误SE
- 估计：Inverse-variance weighting (IVW)

Estimating causal effects. MR estimation with summary level data requires estimates of $\hat{\pi}_l$, the estimated effect of genetic variant l on the exposure with variance $\sigma^2_{x,l}$, and $\hat{\Gamma}_l$, the estimated effect of genetic variant l on the outcome with variance $\sigma^2_{y,l}$. Inverse-variance weight-

$$\hat{\beta}_{\mathrm{IVW}} = \frac{\sum_j w_j \hat{\beta}_j}{\sum_j w_j}, w_j = \frac{\hat{\gamma}_j^2}{\sigma_{Y_j}^2}$$

$$\hat{\beta}_{\mathrm{IVW}} = \frac{\sum_{l=1}^{L} \hat{\pi}_l \hat{\Gamma}_l \sigma_{y,l}^{-2}}{\sum_{l=1}^{L} \hat{\pi}_l^2 \sigma_{y,l}^{-2}}$$

两种理解方法：

- 将使用每个SNP作为IV用比值法估计得到的因果效应估计量 $\hat{\beta}_l = \frac{\hat{\Gamma}_l}{\hat{\pi}_l}$ 用其方差倒数加权
- G-Y关联估计量对G-X关联估计量作回归，权重是G-Y关联估计量方差的倒数

$$\hat{\Gamma}_l = \beta_{\mathrm{IVW}} \hat{\pi}_l + u_l \text{ weighted by } 1/\hat{\sigma}^2_{y,l}$$

IVW估计

$$\hat{\gamma}_j \sim \mathcal{N}(\gamma_j, \sigma_{Xj}^2),\ \hat{\Gamma}_j \sim \mathcal{N}(\Gamma_j, \sigma_{Yj}^2)$$

$$\hat{\beta}_j = \frac{\hat{\Gamma}_j}{\hat{\gamma}_j},$$

$$\mathrm{var}(\hat{\beta}_j) = \mathrm{var}(\frac{\hat{\Gamma}_j}{\hat{\gamma}_j})$$

no measurement error

no sample overlap

$$\approx \frac{\mathrm{var}(\hat{\Gamma}_j)}{{\gamma_i}^2} + \frac{\Gamma_j^2}{\gamma_i^4}\mathrm{var}(\hat{\gamma}_j) - 2\frac{\Gamma_j}{\gamma_j^3}\mathrm{cov}(\hat{\Gamma}_j, \hat{\gamma}_j)$$

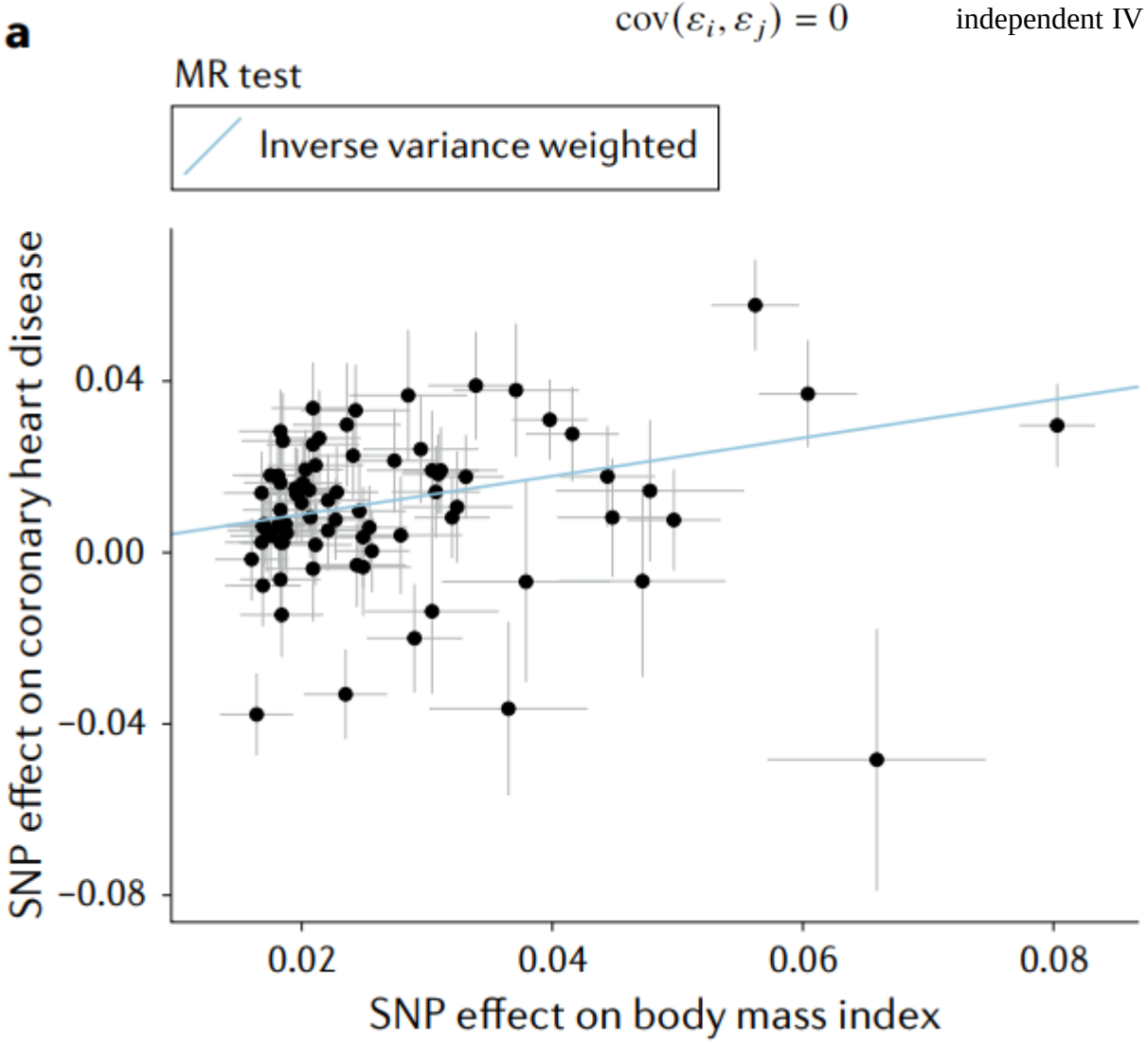
$$\mathrm{var}(\hat{\beta}_j) \approx \frac{\mathrm{var}(\hat{\Gamma}_j)}{{\gamma_j}^2} = \frac{\sigma_{Yj}^2}{{\gamma_j}^2}$$

$$\hat{\mathrm{var}}(\hat{\beta}_j) = \frac{\sigma_{Yj}^2}{\hat{\gamma}_j^2}$$

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

$$\hat{\Gamma}_j = \hat{\gamma}_j \beta_{\mathrm{IVW}} + \varepsilon_j,\ \varepsilon_j \sim \mathcal{N}(0, \sigma_{Yj}^2)$$

$$\mathrm{cov}(\varepsilon_i, \varepsilon_j) = 0$$ independent IV

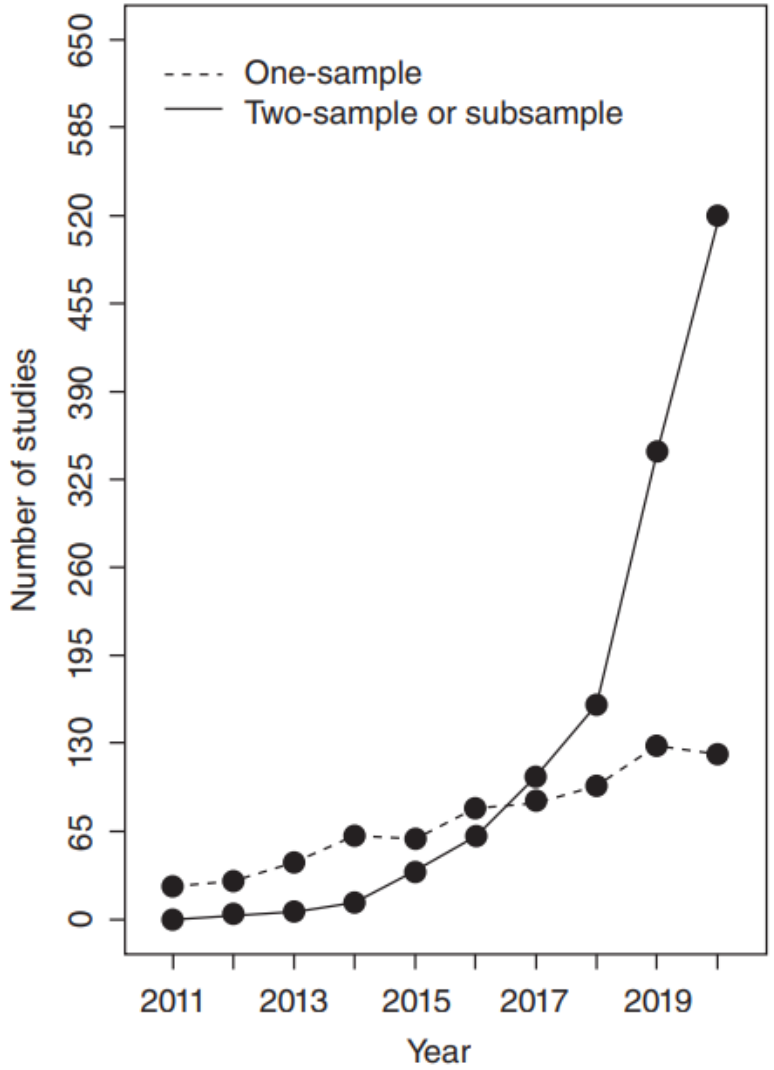


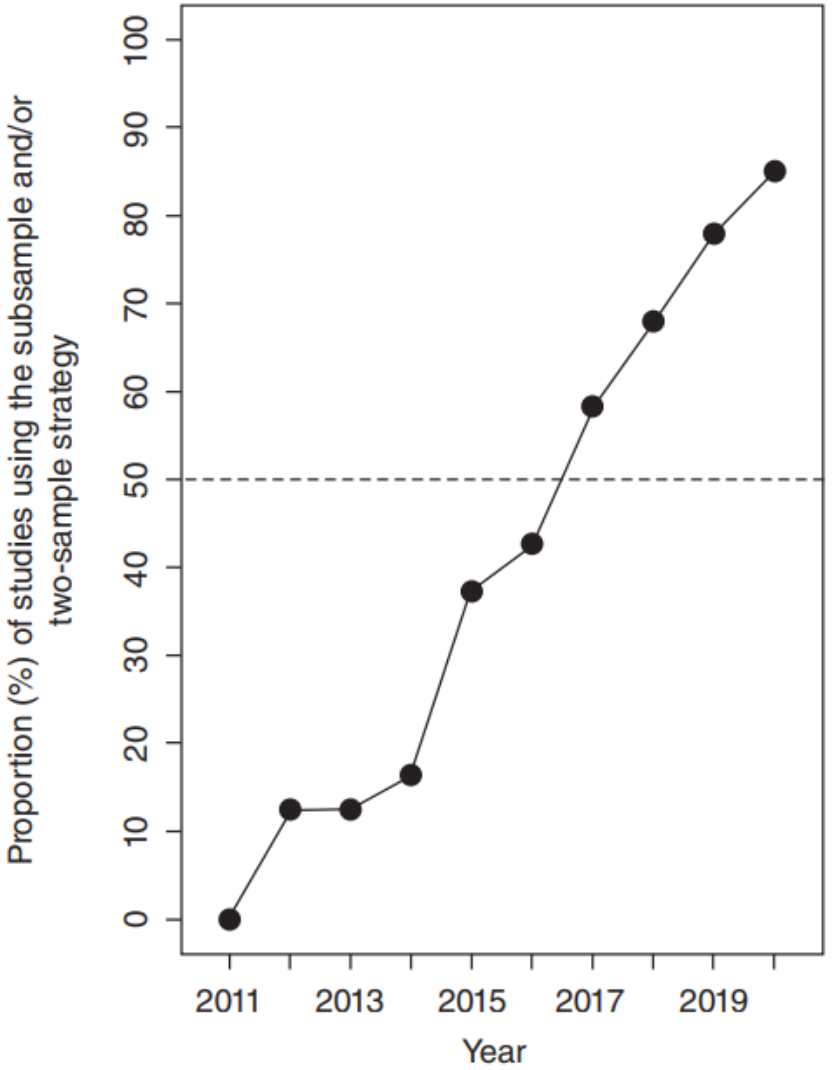
Comparison

Table 3. Comparison of strengths and limitations of one-sample and two-sample Mendelian randomization (MR)

	One-sample MR	Two-sample MR
Strengths	Flexibility of the analytical strategy in terms of regression models that can be performed as well as covariates and participants that can be included/excluded	Improved sample size and power
	Permits thorough evaluation of confounders to test above assumption	Flexibility and enhanced power to perform an array of sensitivity analyses (e.g., pleiotropy-robust methods)
	Allows for comparison with observational estimates in same study (e.g., through Durbin-Wu-Hausman test)	Less time-consuming and easier to implement
	Can model interactions, survival time, and other analyses (including MR analysis of nonlinear effects)	Can evaluate causal relationships between a range of exposure and outcomes, which might not be possible in a single sample setting
		Unable to thoroughly evaluate individual-level confounding factors
		Assumes the two samples are exchangeable. Examples of where this is difficult to assert are where the samples are heterogeneous in terms of age, sex distributions or ancestry
Limitations	Traditionally low power and therefore imprecise causal estimates	Potential for selection bias caused by study sampling
		Weak instrument bias is toward null
	Potential for selection bias caused by study sampling	Winner's curse in which the discovery GWAS used to estimate the SNP-trait association may overestimate the effect of the genetic instrument relative to the exposure
	Weak instrument bias is toward observational estimate	Relative rigidity of the summary data available, which is limited by the original GWAS model performed (e.g., adjustment for unwanted covariates and a lack of available data on subgroups of interest (e.g., sex-specific estimates)
	Winner's curse in which the sample in the discovery GWAS is the same as that used for MR, which can lead to overestimation of the strength of association of the genetic instrument with the exposure	SNP–exposure and SNP–outcome associations should be coded relative to the same effect allele, also known as "harmonization," which is nontrivial in the situation of palindromic SNPs (i.e., G/C and A/T SNPs) and in the absence of information on allele frequencies
	Need to have access to individual-level genetic and phenotypic data	Assumes no overlap between samples, which could bias estimates if this is not true
		Direct comparison with observational estimates not as straightforward
		Unable to model interactions, survival time, and other analyses (including nonlinear analyses)

Trend


One-sample
Two-sample or subsample
Number of studies
0
65
130
195
260
325
390
455
520
585
650
2011
2013
2015
2017
2019
Year


Proportion (%) of studies using the subsample and/or two-sample strategy
0
10
20
30
40
50
60
70
80
90
100
2011
2013
2015
2017
2019
Year

Binary outcome

- Individual-level data:
  - 2SPS: two-stage predictor substitution

$$\widehat{x}_{es} = r_s(w\widehat{\alpha}_s) \text{for} s = 1, \ldots, S \qquad y = M(x_o\gamma_o + \widehat{x}_e\gamma_e) + e^{2SPS}$$

GLM (generalized linear model): LM + link function

  - 2SRI: two-stage residual inclusion

$$\widehat{x}_{us} = x_{es} - r_s(w\widehat{\alpha}_s) \text{for} s = 1, \ldots, S \qquad y = M(x_e\beta_e + x_o\beta_o + \widehat{x}_u\beta_u) + e^{2SRI}$$

- Summary data:
  - Logistic regression: $\beta_{YZ} = \log OR$
  - Odds ratio

Binary outcome

2SPS

2SRI

Table 1. Different Instrumental Variable Estimators and the Assumptions Required for Consistency

Estimator (Equation No.)	Target Parameter	Assumptions Required for Consistency
Ratio estimator (5)	Population CRR	Model for *Y* given do(*X*) and *U* is log-linear in *X* and *U*, without interaction; model for *X* given *Z* and *U* is linear without interaction, and *X* is approximately normally distributed (see reference 13).
Ratio estimator (6)	Population COR	Not generally consistent; approximately consistent for rare diseases under same assumptions as ratio estimator of the population CRR.
2-stage, logistic second stage (7, 8)	Population COR	Same as ratio estimator of population COR.
2-stage, log-linear second stage	Population CRR	Same as ratio estimator of population CRR.
Control function, logistic second stage (9, 10)	COR conditional on *U*	Generally not consistent, but converges to LSMM when *X* is normally distributed (see reference 47).
Control function, log-linear second stage	Population CRR	Same as 2-stage estimator with log-linear second stage.
MSMM (12)	CRR effect on exposed	Log-linear model for *Y* given do(*X*), *X* and *Z*, no effect modification by *Z*.
MSMM (12)	Population CRR	Log-linear model for *Y* given do(*X*) and *U*, no effect modification by *U*.
LSMM (13, 14)	COR effect on exposed	Logistic model for *Y* given do(*X*), *X* and *Z*, no effect modification by *Z*; association model for *Y* given *X* and *Z* has intercept, unrestricted main effect of *Z* and fitted by maximum likelihood.
MGMM (15, 16)	Population CRR	Same as MSMM estimator of the population CRR.

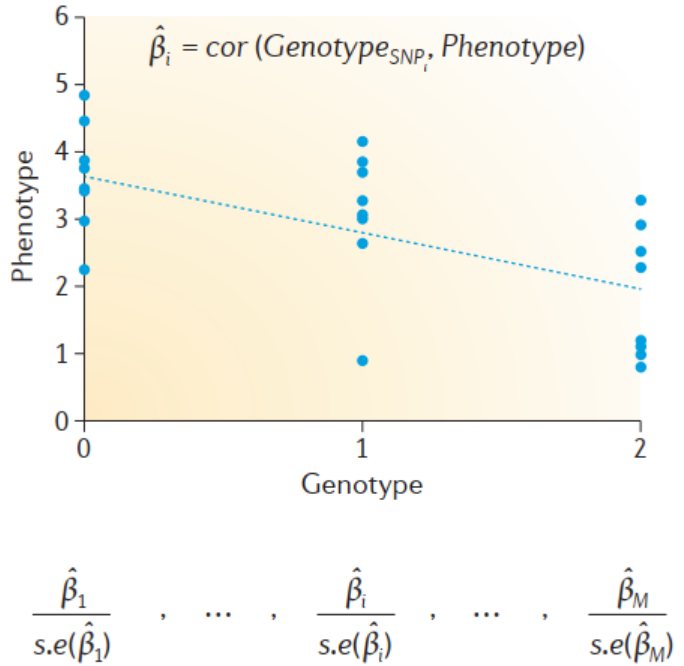
Abbreviations: COR, causal odds ratio; CRR, causal risk ratio; LSMM, logistic structural mean model; MGMM, multiplicative generalized method of moments; MSMM, multiplicative structural mean model.

MR设计的扩展

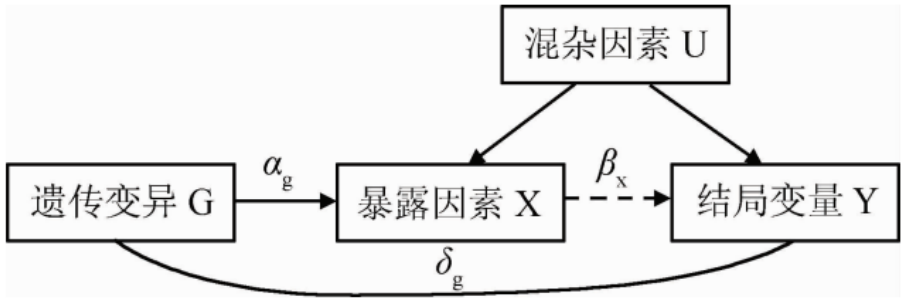
Recall

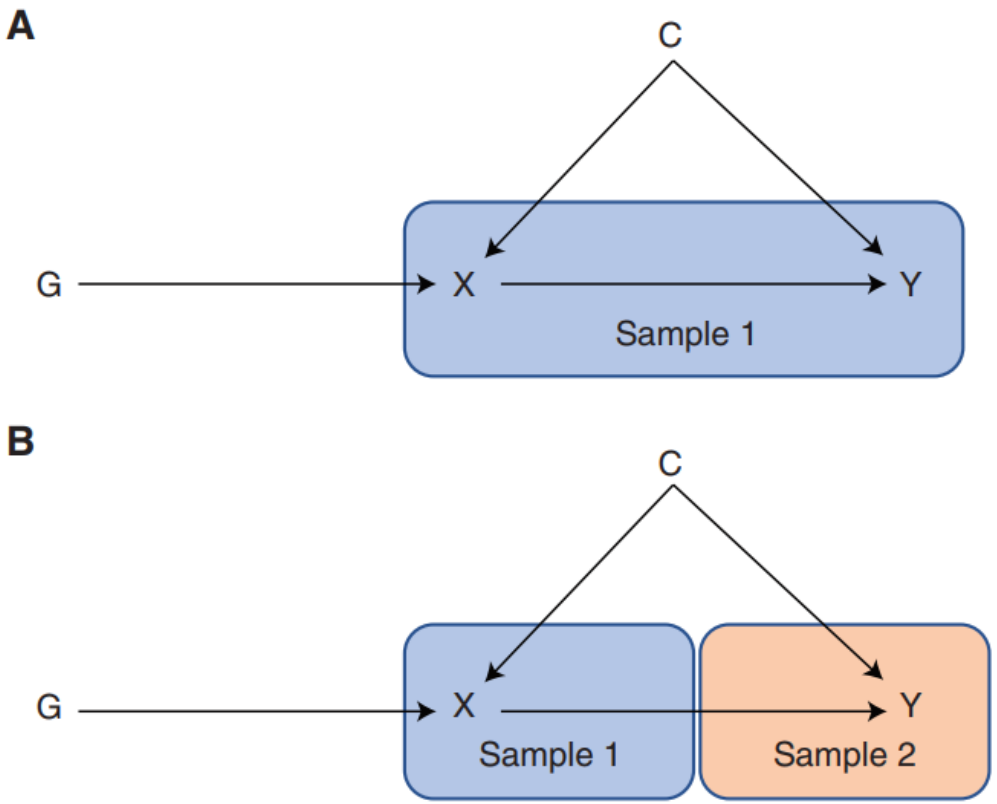
	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6
Person 1	G	T	G	A	A	T
Person 2	G	T	C	C	T	C
Person 3	C	A	G	C	A	C
Person 4	C	A	C	C	T	C

Individual-level data

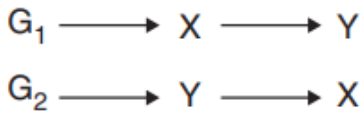
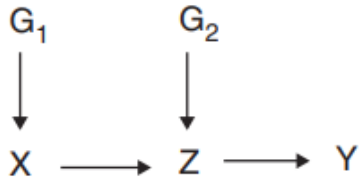
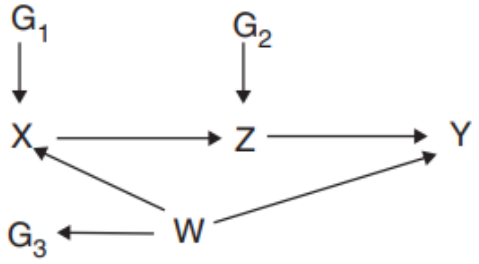


Summary data

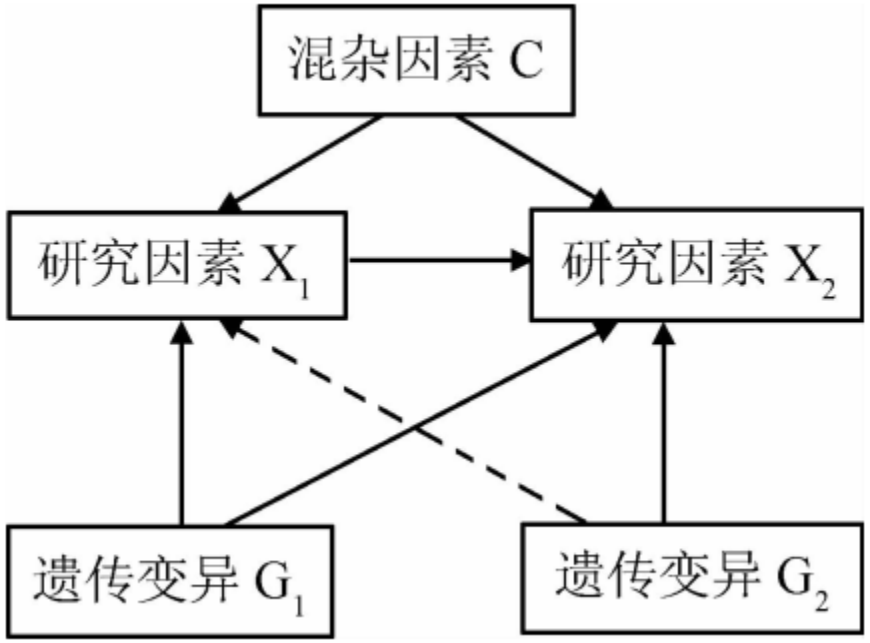




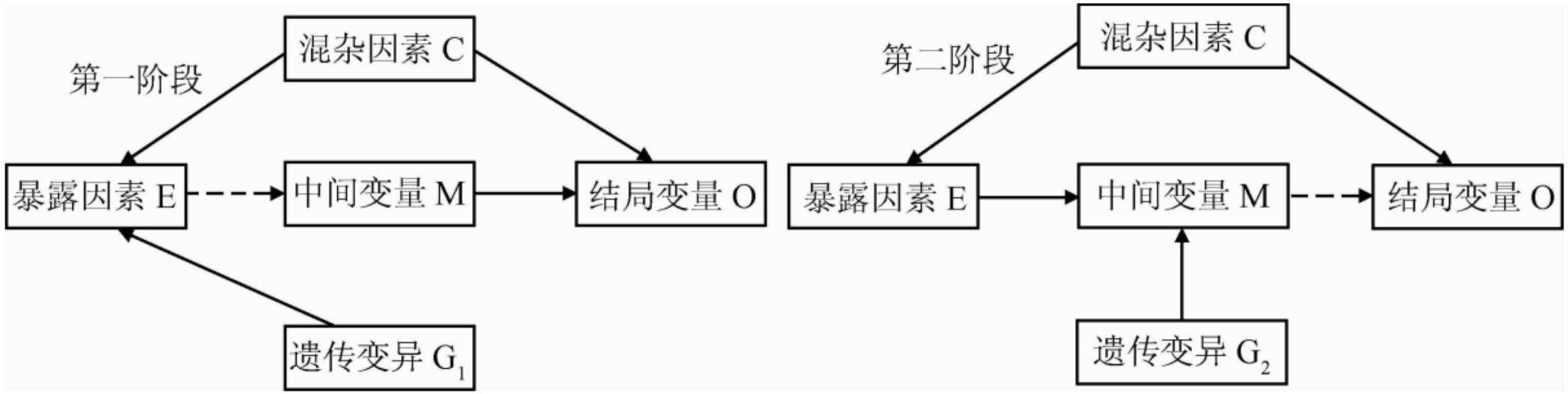
Extension

Method	Description	Directed acyclic graphs (DAGs)	Applications
Bidirectional or reciprocal MR (Timpson et al. 2011)	Used to evaluate the causal direction(s) of effect between two traits X and Y, with the use of valid instruments G_X and G_Y		Body mass index (BMI) and vitamin D (Vimaleswaran et al. 2013)
Two-step MR (Relton and Davey Smith 2012)	Used to assess the role of an intermediary factor (Z) in mediating the effect of X on Y with the use of valid instruments G_X and G_z		DNA methylation, gene expression, and BMI (Mendelson et al. 2017)
Network MR (Burgess et al. 2015)	Extension of the two-step MR approach to consider the causal role of multiple mediators or causal networks		Effect of education on cardiovascular disease (CVD) via smoking, BMI, and alcohol (Carter et al. 2019)

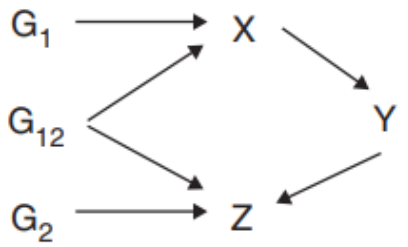
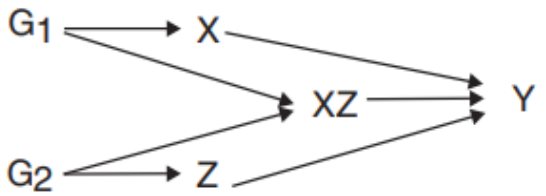
Reciprocal MR: reverse causality



Two-step MR: mediation effect

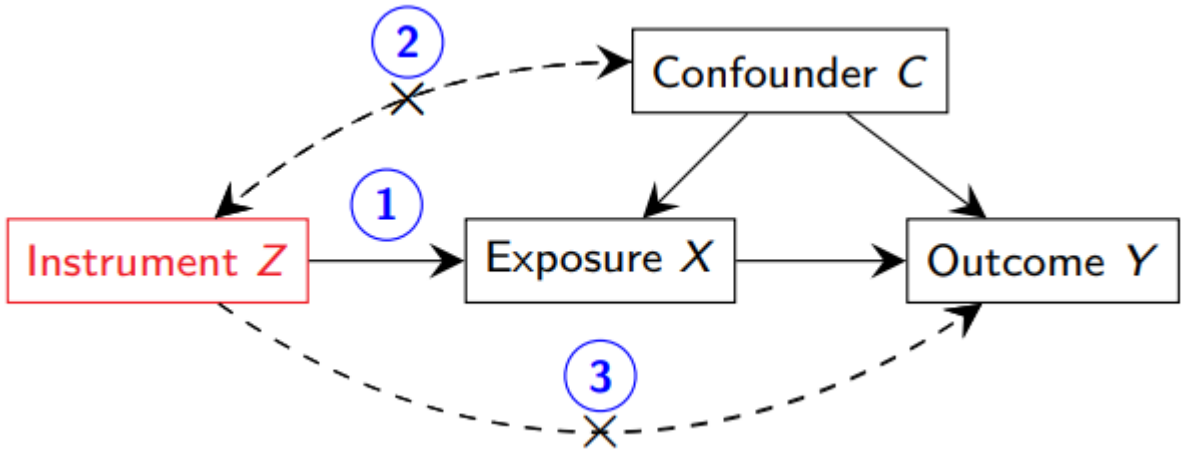


Extension

Multivariable MR (Burgess and Thompson 2015)	Used to assess the role of multiple correlated exposures using genetic variants that are associated with one or multiple exposures to estimate the independent causal effect of each exposure on the outcome. Can also be adapted to evaluate mediation (in combination with or separate to two-step MR)		Lipid fractions and coronary heart disease (CHD) (Burgess et al. 2014)
Factorial MR/ exposure interactions (Rees et al. 2020)	Used to determine the combined causal effects of two or more risk factors for disease within a factorial design		Statin (HMGCR), ezetimibe (NPC1L1) and CHD (Ference et al. 2015)

违背MR基本假设的情况及处理

Recall the assumptions



1. **Relevance**: *Z* is associated with the exposure (*X*).
2. **Exchangeability**: *Z* is independent of the unmeasured confounder (*C*).
3. **Exclusion restriction**: *Z* cannot have any direct effect on the outcome (*Y*).

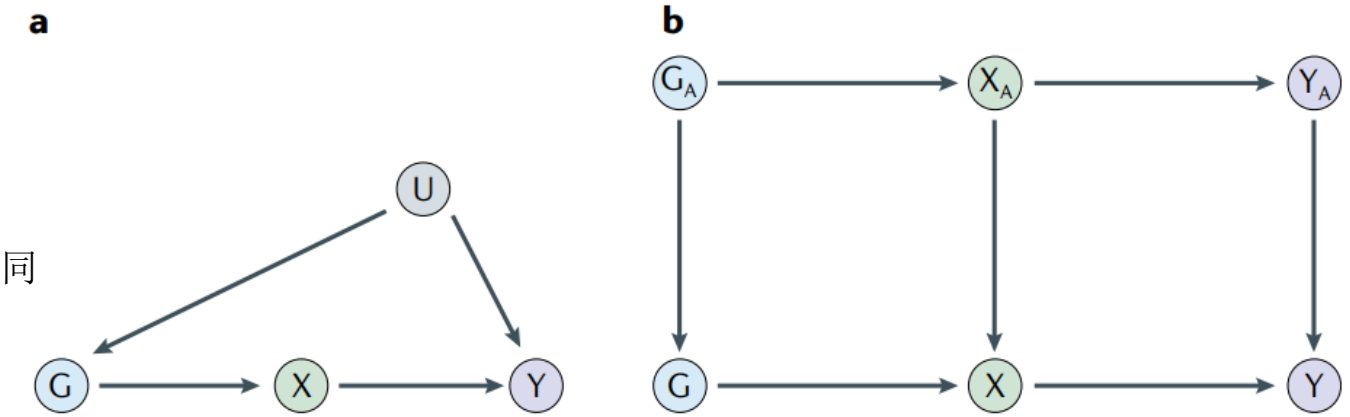
I. Relevance: weak instrument

- 单个SNP的遗传力很小
- Weak IV：在one-sample MR中常常高估X-Y的effect，在two-sample MR中常常低估X-Y的effect
- 使用更多SNP作为IV，或者使用PRS：polygenic MR
- 评价IV强度：first-stage F statistic (*F* >10)

II. Exchangeability

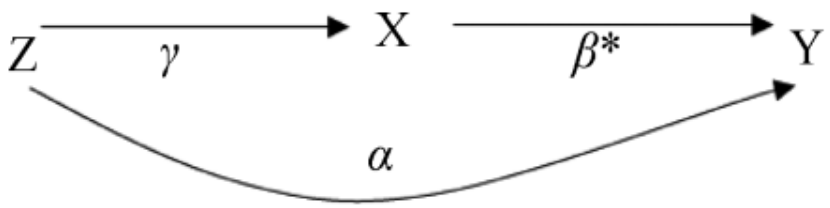
- Population-based MR ≠ RCT
- instrument–outcome confounding:
  - Population stratification
  - Dynastic effects
  - Assortative mating
  - Transmission ratio distortion (TRD): 亲本的2个等位基因遗传给后代的概率不同
    - meiotic drive 减数分裂驱动
    - gametic competition 配子竞争
    - embryo lethality 胚胎致死

Dynastic effects 世代效应



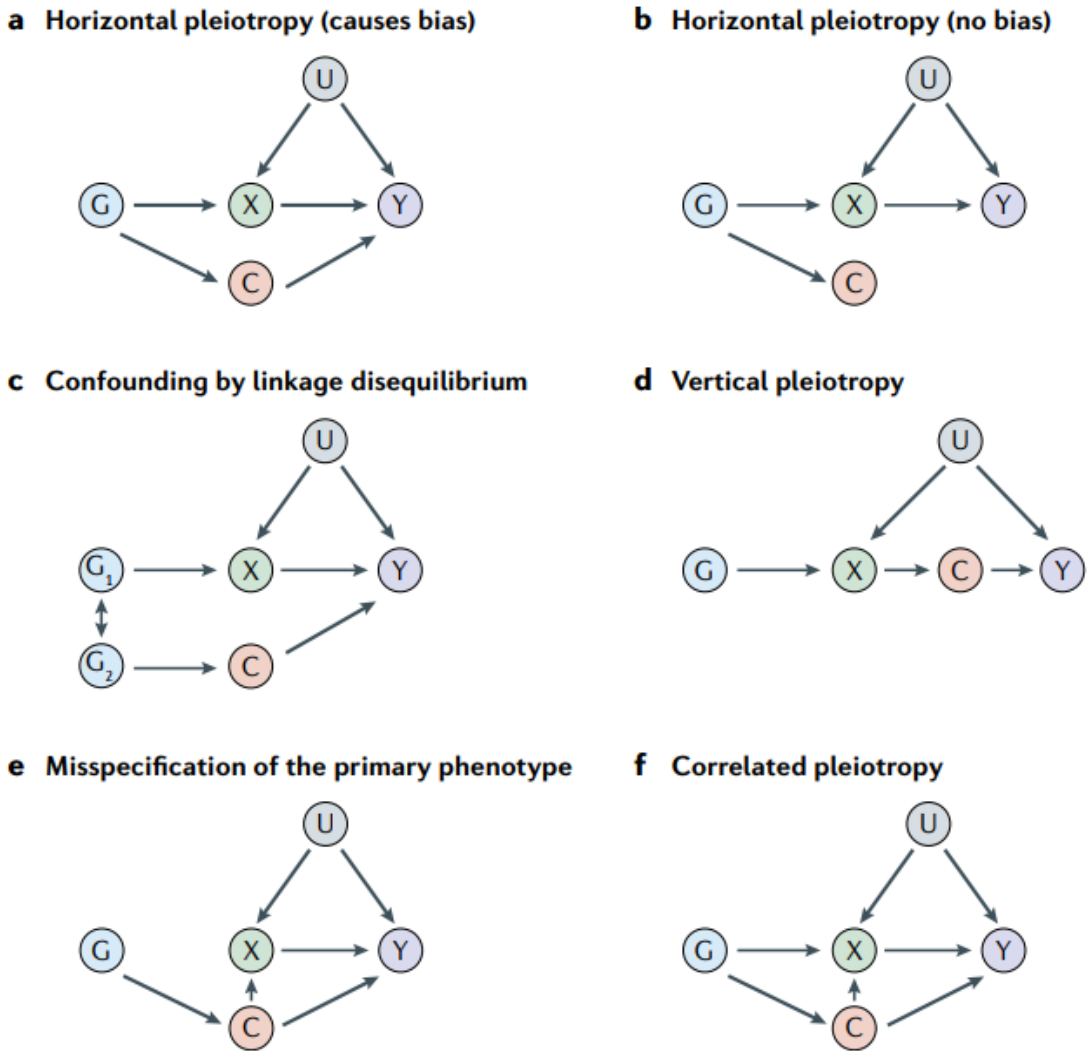
III. Exclusion restriction: invalid IV

- Pleiotropy & linkage disequilibrium



$$Y_i = z_i\tau + \varepsilon, \tau_j = \alpha_j + \gamma_j\beta^*$$

$$\hat{\beta}_j = \frac{\tau_j}{\hat{\gamma}_j} = \frac{\alpha_j + \hat{\gamma}_j\beta^*}{\hat{\gamma}_j} = \beta^* + \frac{\alpha_j}{\hat{\gamma}_j} = \beta^* + b_j(\text{偏倚项})$$



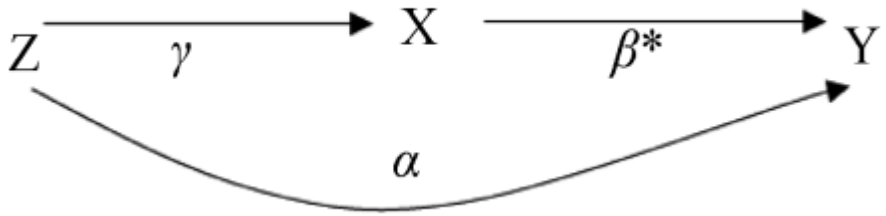
处理多效性的方法

- 多效性的类别：
  - 水平多效性（horizontal pleiotropy）、垂直多效性（vertical pleiotropy）
  - 平衡多效性（balanced pleiotropy）、有向多效性（directional pleiotropy）
  - 无关多效性（uncorrelated pleiotropy）、相关多效性（correlated pleiotropy）

- 两个基本思路：

1. 识别多效性IV（outlier）并将其排除
2. 建模多效性的影响，校正因果估计值

检验/识别多效性



- 过度识别检验：检验各个IV得到的因果估计量的异质性
- 2SLS: Sargan's test
- IVW: Cochran's Q test; I^2 statistics

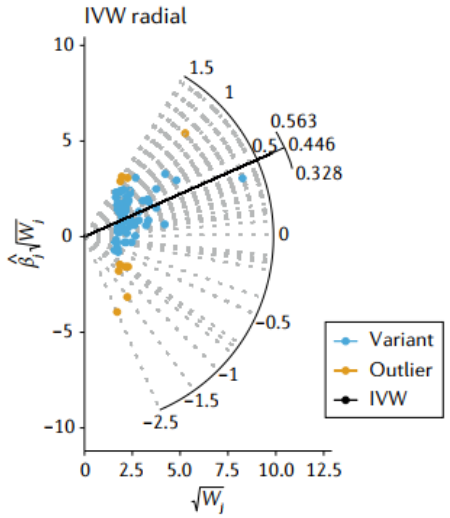
$$Q = \sum_j Q_j = \sum_j w_j \left(\hat{\beta}_j - \hat{\beta}\right)^2$$

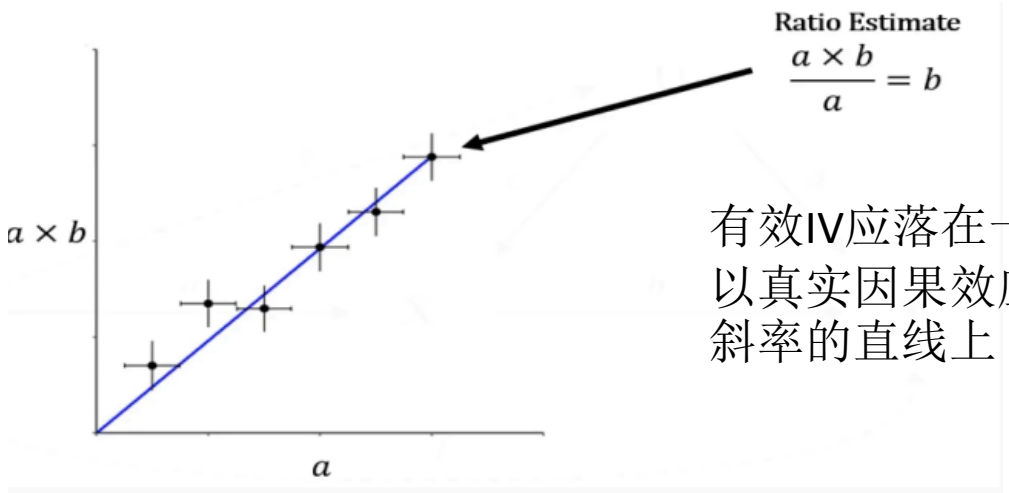
- scatter plots

IV都有效：对所有Z都有α=0

$$Y_i = z_i\tau + \varepsilon, \tau_j = \alpha_j + \gamma_j\beta^*$$

$$\hat{\beta}_j = \frac{\tau_j}{\hat{\gamma}_j} = \frac{\alpha_j + \hat{\gamma}_j\beta^*}{\hat{\gamma}_j} = \beta^* + \frac{\alpha_j}{\hat{\gamma}_j} = \beta^* + b_j(\text{偏倚项})$$





有效IV应落在一条以真实因果效应为斜率的直线上

处理多效性

- One-sample
  - Lasso-type: sisVIVE, post-adaptive lasso
  - hard thresholding with voting (TSHT)
  - Confidence Interval Method (CIIV)
  - constrained IV, MR-GxE
- Two-sample
  - MR-Egger
  - Median-based
  - Mode-based
  - Robust regression, penalization
  - Bayesian MR, BWMR, JAM-MR
  - CAUSE
  - MR-PRESSO, MR-link, MRmix, MRAID

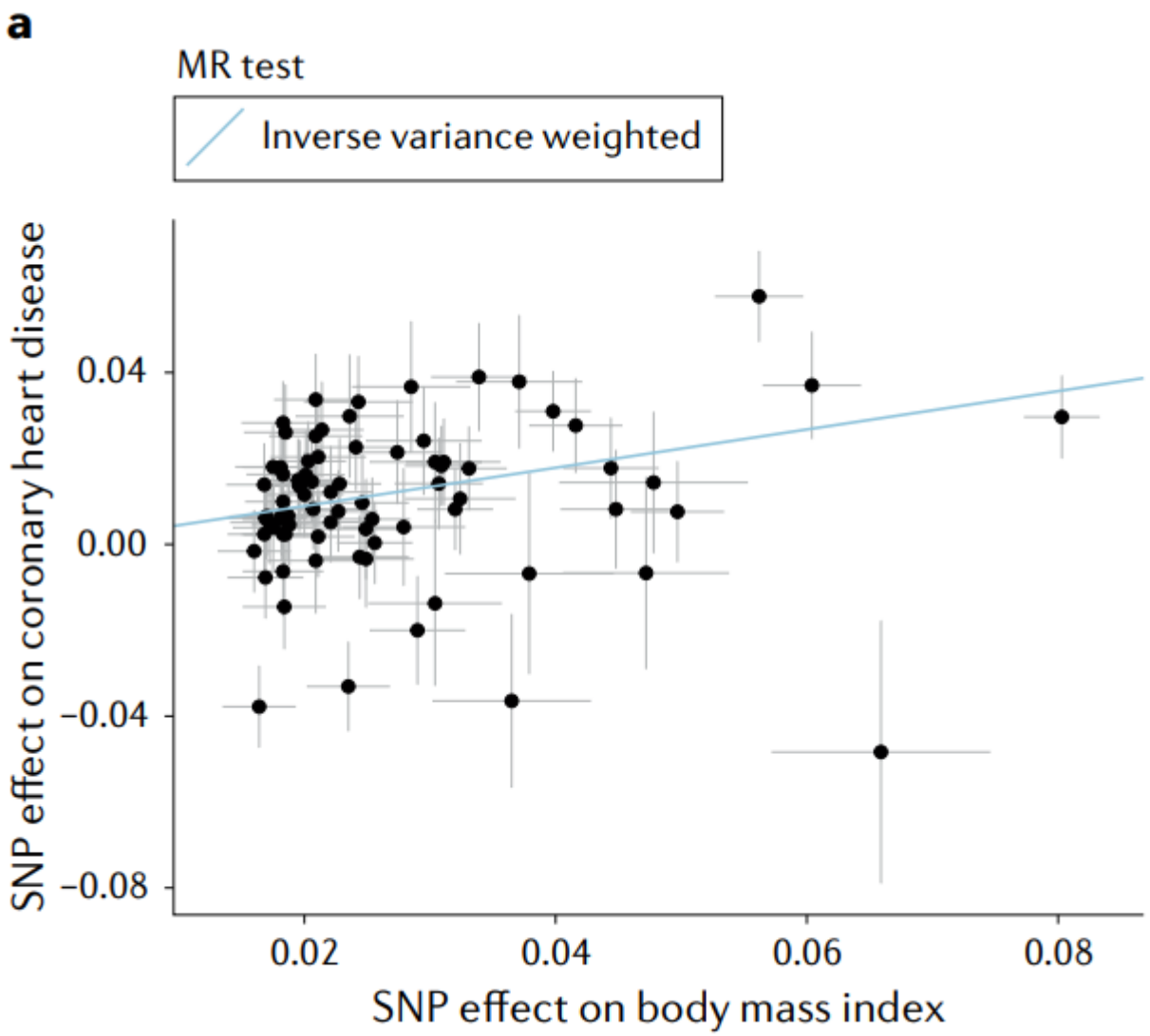
$$(\hat{\delta}, \hat{\beta}) = \arg\min_{\delta,\beta} \frac{1}{2}||\boldsymbol{P}_Z(\boldsymbol{Y} - \boldsymbol{Z}\delta - \boldsymbol{X}\beta)||_2^2 + \lambda||\delta||_1$$

Recall: IVW

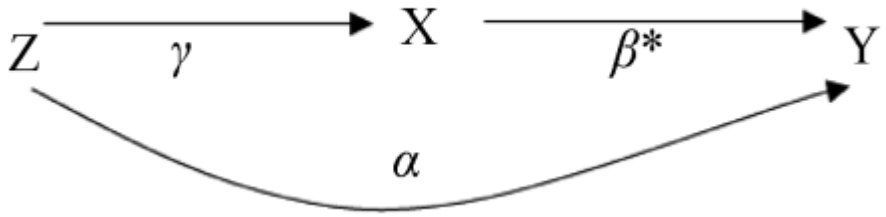
$$\widehat{\beta}_{\mathrm{IVW}} = \frac{\sum_{l=1}^{L} \widehat{\pi}_l \widehat{\Gamma}_l \sigma_{y,l}^{-2}}{\sum_{l=1}^{L} \widehat{\pi}_l^2 \sigma_{y,l}^{-2}}$$

- 第二种理解方法

$\widehat{\Gamma}_l = \beta_{\mathrm{IVW}} \widehat{\pi}_l + u_l$ weighted by $1/\widehat{\sigma}_{y,l}^2$

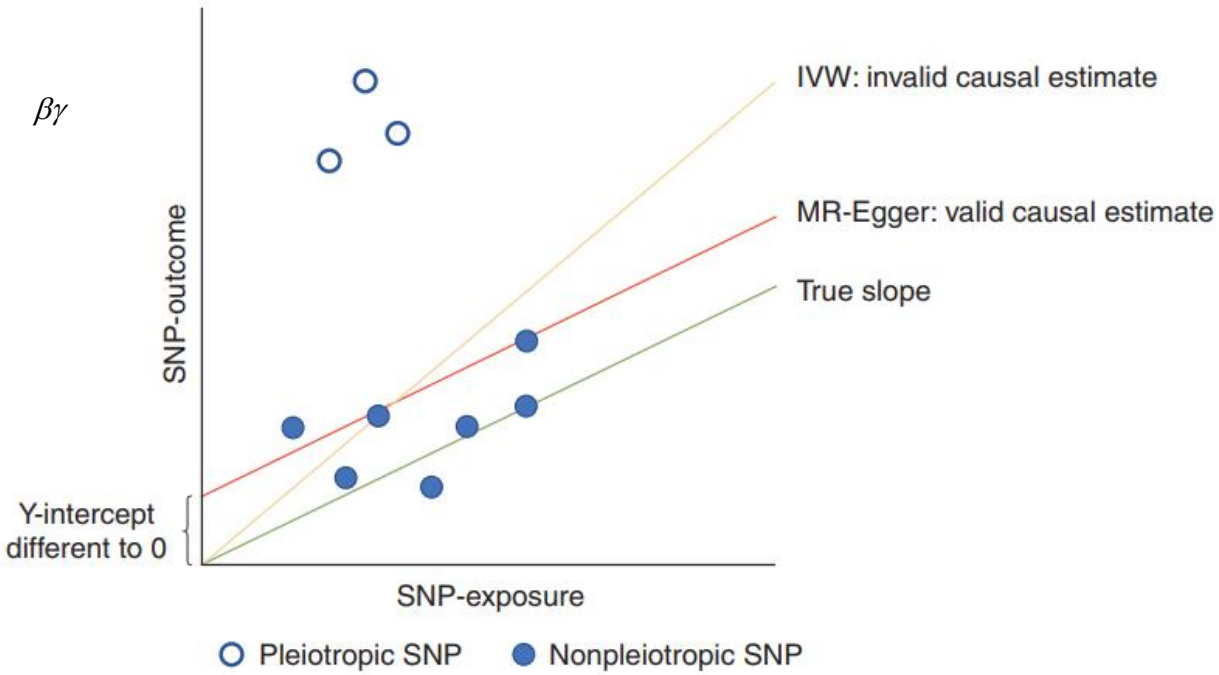


From IVW to MR-Egger



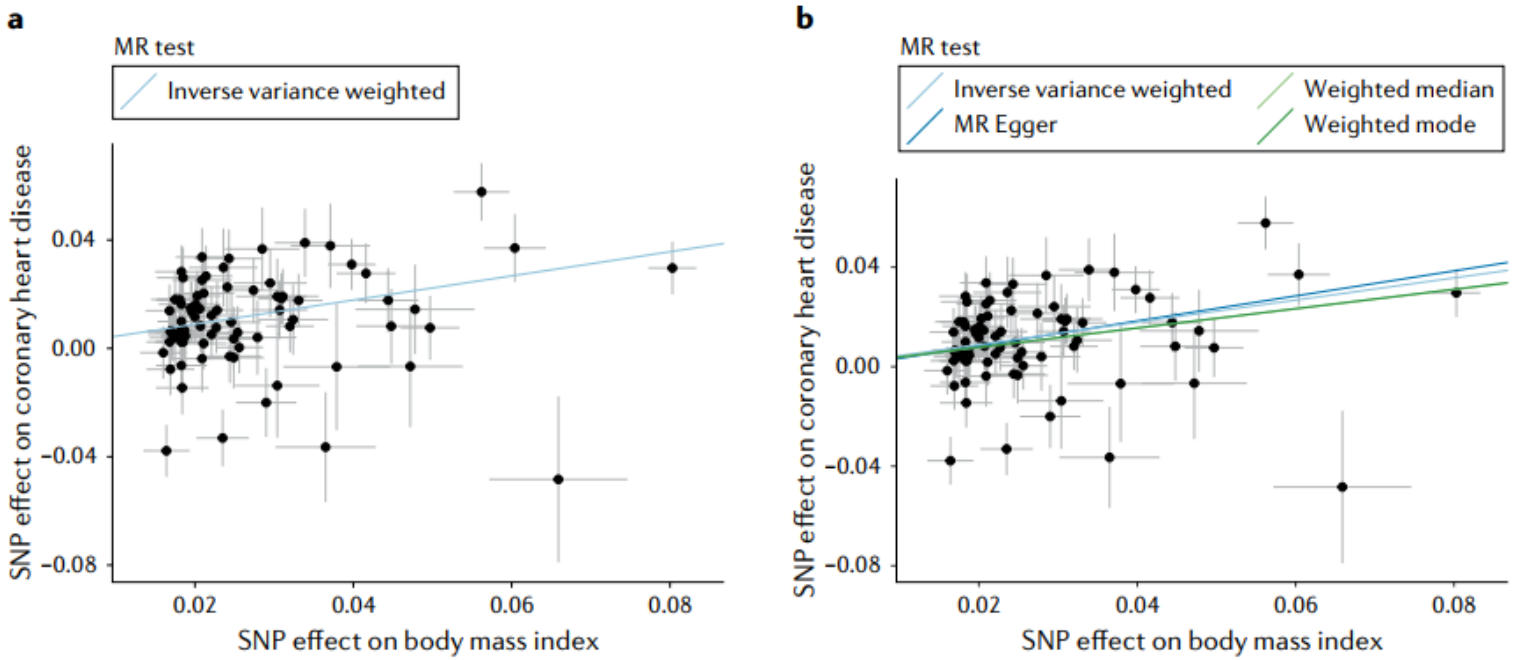
- MR-Egger：
  IVW在回归时假定截距为0，MR-Egger则可以有截距项(average directional pleiortopy)
- 需要额外假设InSIDE条件：G-X-Y的路径（$\beta\gamma$）与G通过多效性直接影响Y的路径（α）相互独立 [the instrument strength independent of direct effect]

$$\Gamma_j = \alpha_j + \beta\gamma_j \qquad \hat{\Gamma}_j = \beta_{0E} + \beta_E\hat{\gamma}_j$$



Median and mode estimator

- Median：假定超过一半的IV是有效的（过半多数）
- Mode：假定所有得到相同估计值的组中，包含IV个数最多的那一组是有效的（简单多数）
- 加权版本



Regularization methods

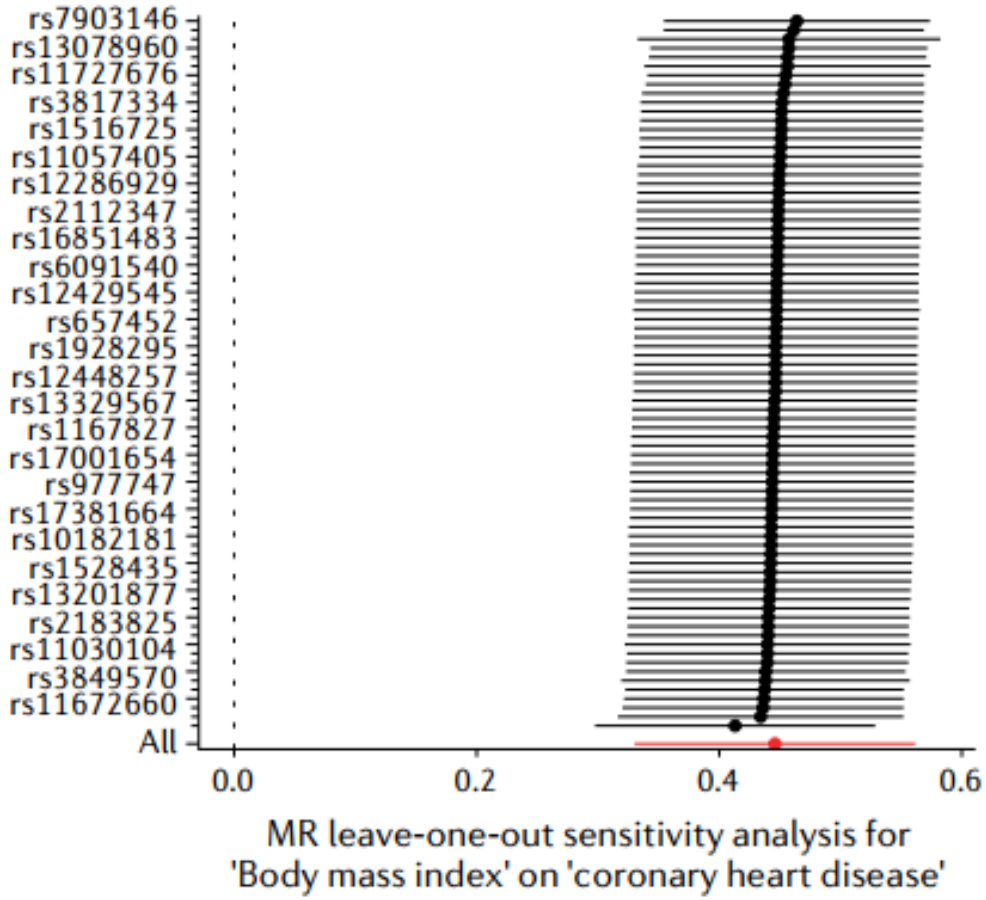
- Recall IVW: $\hat{\beta}_{Yj} = \theta\,\hat{\beta}_{Xj} + \epsilon_j, \quad \epsilon_j \sim \mathcal{N}[0, \mathrm{se}(\hat{\beta}_{Yj})^2],$
  - 最小化平方误差 MSE $\hat{\theta}_{IVW} = \arg\min_{\theta} \sum_i \mathrm{se}(\hat{\beta}_{Yj})^{-2}(\hat{\beta}_{Yj} - \theta\,\hat{\beta}_{Xj})^2.$
  - MR-Egger: $\hat{\beta}_{Yj} = \theta_0 + \theta_E\,\hat{\beta}_{Xj} + \epsilon_j, \quad \epsilon_j \sim \mathcal{N}[0, \mathrm{se}(\hat{\beta}_{Yj})^2].$
- Pleiotropy: add penalized term
  - L1-penalty

$$\hat{\theta}_{\mathrm{LASSO},\lambda} = \arg\min_{\theta} \left[\sum_j \mathrm{se}(\hat{\beta}_{Yj})^{-2}(\hat{\beta}_{Yj} - \theta_{0j} - \theta_L\,\hat{\beta}_{Xj})^2 + \lambda \sum_j |\theta_{0j}| \right],$$

- Bayesian:
  - Penalty term → shrinkage prior (e.g. horseshoe, spike-slab, etc.)

Sensitivity analysis

- Heterogeneity test
  - Cochran's Q test; I^2 statistics
- Pleiotropy test
  - MR-Egger截距项： test directional pleiotropy
  - MR-PRESSO test
- 留一法 leave-one-out
- Funnel plot: 对称性



Summary data methods for independent IV

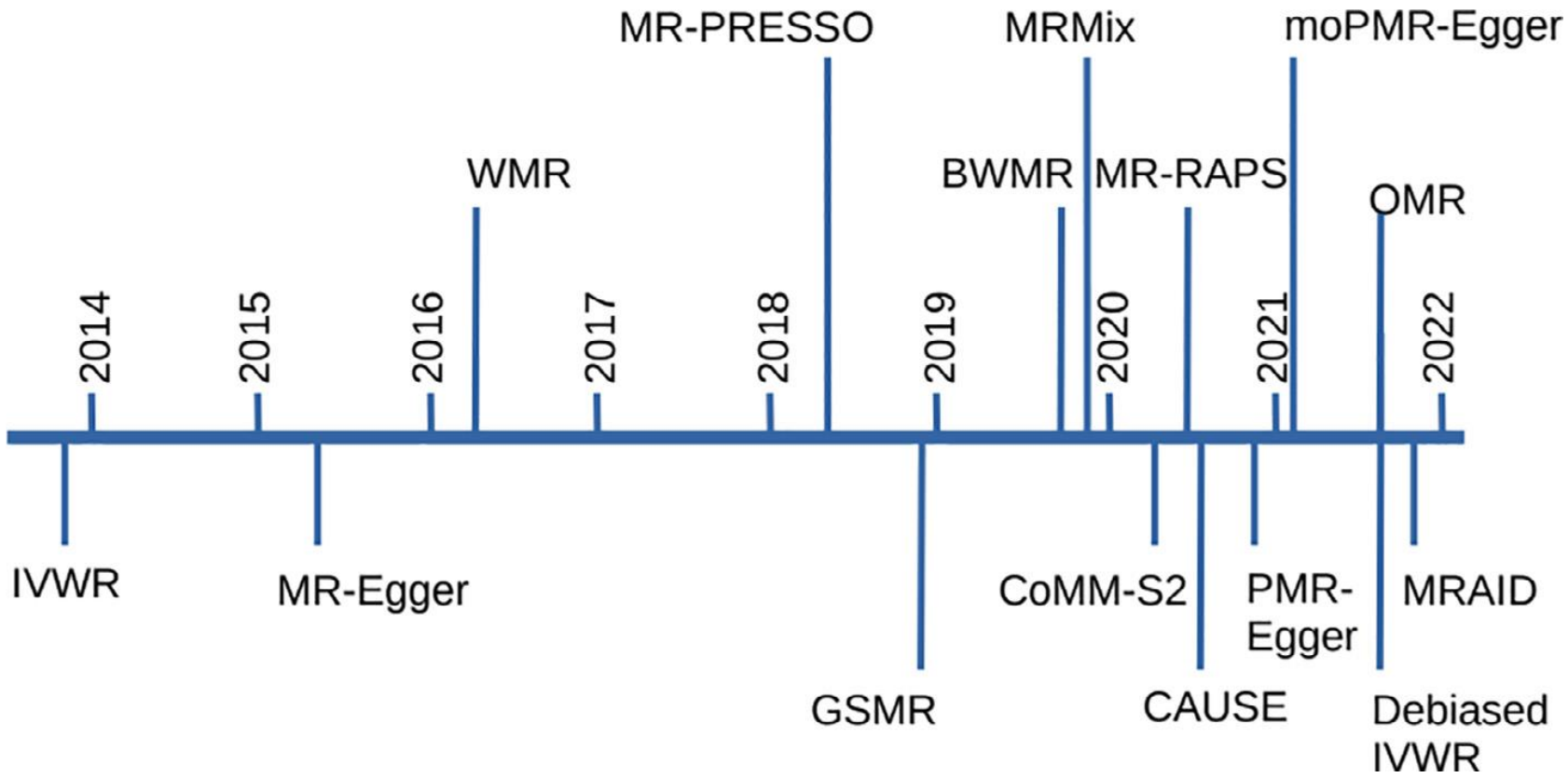
Approach	Assumption	Description	References
Inverse-variance weighted (IVW) and MR-Egger regression	No measurement error (NOME)	There is no measurement error in the association between the single-nucleotide polymorphism (SNP) and the exposure	Bowden et al. 2016b
MR-Egger regression	Instrument strength independent of direct effect (InSIDE)	The strength of the SNP–exposure association should not correlate with the strength of the pleiotropy effect	Bowden et al. 2015
Modal estimator	Zero modal pleiotropy assumption (ZEMPA)	The most common causal effect estimate is a consistent estimate of the true causal effect, even if the majority of instruments are invalid	Hartwig et al. 2017
Median estimator	Simple median = the causal effect is provided by the median SNP estimate	Simple median = at least 50% of the instruments are valid (i.e., not pleiotropic)	Bowden et al. 2016a
	Weighted median = the causal effect is provided by the weighted median SNP estimate	Weighted median = at least 50% of the weight in the analysis stems from variants that are valid instruments (i.e., not pleiotropic)	

这里几种方法都隐含了IV独立性的假定，对于cis-MR等使用很多处于LD状态下、不独立的IV，需要使用其他方法（如penalized regression，PCA等）处理

Other MR methods

Category	Core IV assumption relaxed	Individual-level data	Summary data
'Basic' MR method	None	Wald ratio estimation, 2SLS regression analysis[a]	Wald ratio estimation, IVW[a,37]
Weak instrument robust methods	IV1; allows for weak instruments	LIML[26], allele score approaches[26]	MR RAPS[87], debiased IVW[187], MR GRAPPLE[88], NOME adjustment[188], two-sample AR[189]
Outlier/variant selection and removal	IV3; allows for balanced/sparse pleiotropy	Weighted median[190]	Weighted median[a,82]
Outlier/variant selection and removal	IV3; allows for (some) directional pleiotropy	sisVIVE[70], adaptive LASSO[71], weighted mode[190]	Weighted mode[a,83], MR LASSO[84], Steiger filtering[a,93], Welch-weighted Egger[94], contamination mixture[191], GSMR[79], MR-Clust[192], Bayesian MIMR[193], CIV[72]
Outlier/variant adjustment	IV3; allows for balanced pleiotropy	Limited approaches currently available	MR RAPS[87], MRCIP[194]
Outlier/variant adjustment	IV3; allows for (some) directional pleiotropy	Limited approaches currently available	MR TRYX[85], MR Robust[84], MR CAUSE[89], MR PRESSO[86], MR GRAPPLE[88], MRMix[195], MR-LDP[196], IMRP[197], regularization[198], MR-PATH (see preprint[199])
Estimation adjustment	IV3; allows for balanced pleiotropy	Limited approaches currently available	Debiased IVW[187]
Estimation adjustment	IV3; allows for (some) directional pleiotropy	Constrained IVs[72], multivariable MR[73]	MR Egger[90], multivariable MR[73,91], MR Link[200], hJAM[201], GIV[202], Bayesian network analysis[203], BMRE[204], BayesMR[205]
Environmental control adjustment	IV3; allows for (some) directional pleiotropy	MR GxE[75,76], MR GENIUS[77]	Limited approaches currently available

MR methods for summary data



Software

- 代码演示
  - *AER*

$$\hat{\beta}_{IV} = (Z'X)^{-1}Z'Y$$

$$\beta_{2SLS} = (\hat{X}'\hat{X})^{-1}\hat{X}'Y = (P_Z'X'P_ZX)^{-1}P_Z'X'Y,$$

$$\hat{\beta}_{2SLS} = (X'P_ZX)^{-1}X'P_ZY = [X'Z(Z'Z)^{-1}Z'X]^{-1}X'Z(Z'Z)^{-1}Z'Y$$

  - *MendelianRandomization*
  - *TwoSampleMR*

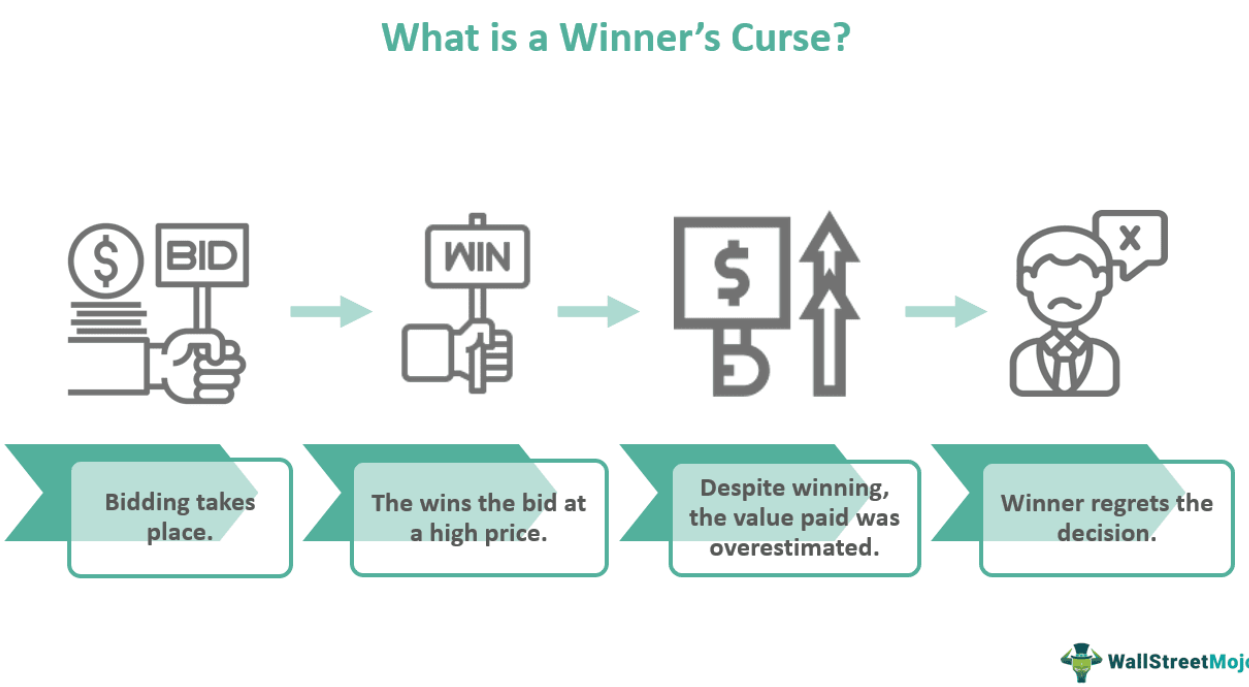
Package name	Software	Description
Individual-level data		
AER	R	Includes the ivreg function for 2SLS estimation
OneSampleMR	R	Various functions for one-sample IV analyses, including the Sanderson–Windmeijer *F* statistic, and various estimators (two-stage predictor substitution, two-stage residual inclusion, structural mean models)
ivmodel	R	Various functions for individual-level IV analyses, includes LIML, weak instrument tests and sensitivity analyses
ivtools	R	Various functions for individual-level IV analyses, including functions to fit structural mean models
ivonesamplemr	Stata	Includes various estimators (two-stage predictor substitution, two-stage residual inclusion, structural mean models) for one-sample IV analyses
ivreg2	Stata	Stata module for extended IVs/2SLS and generalized method of moments estimation
ivregress	Stata	Linear IV estimators including 2SLS
Summary-level data		
MendelianRandomization	R	Implements several methods for performing MR analyses with summarized data and an interface with the PhenoScanner database
TwoSampleMR and MR-Base app	R/web-app	MR-base is an analytical platform for MR. TwoSampleMR is the R package providing the functions to perform MR estimation. Both are linked to the OpenGWAS project, a large database of GWAS summary statistics
mrrobust	Stata	Provides various programs for two-sample MR analyses in Stata

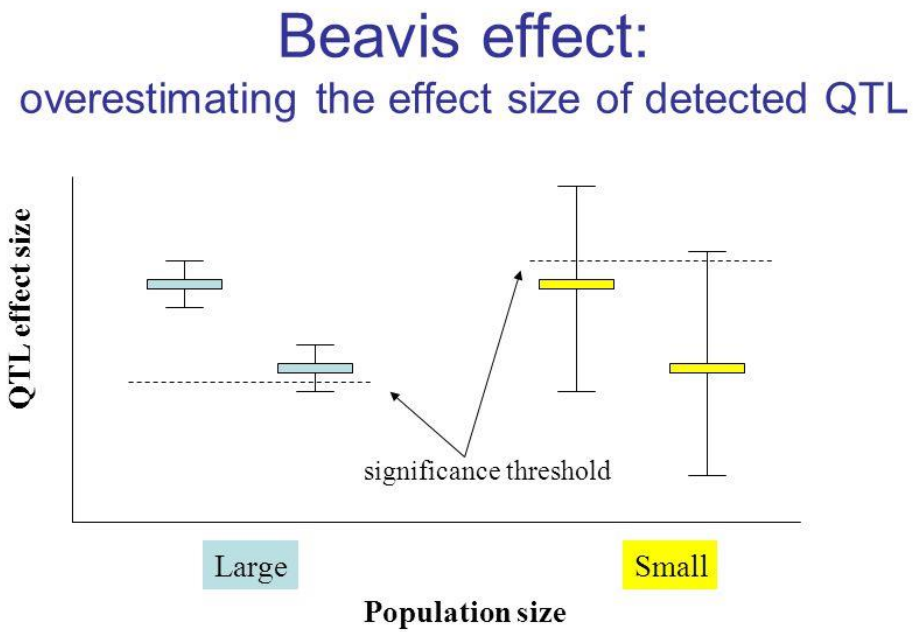
MR研究的局限性

Limitations

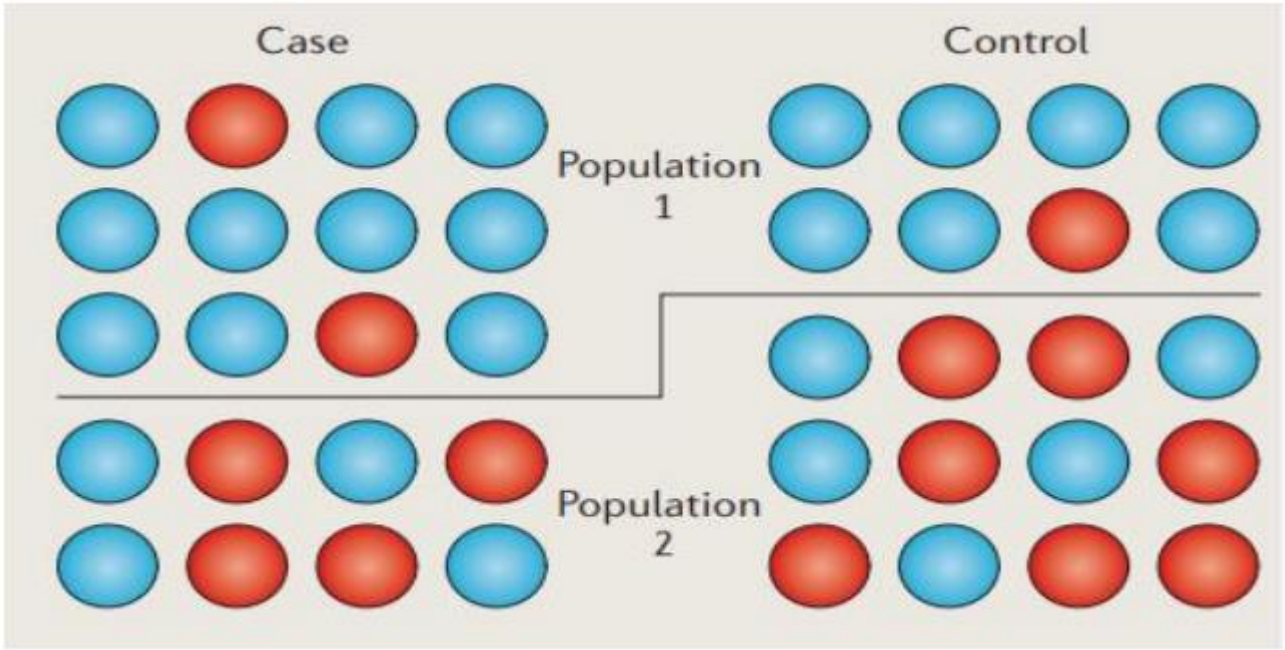
- IV selection: strength, pleiotropy, linkage
- Interpretation: 非遗传因素决定的暴露变化对结局的影响 Gene–environment equivalence
- Non-linear: model misspecification
- Beavis effect: winner’s curse
- Population stratification & Selection bias
- Time-varying exposure: 发育代偿机制
- Gene-gene interaction & Gene-environment interaction
- Reverse causality
- Collider bias

Winner's curse





Population stratification



1/6

6/12

1/8

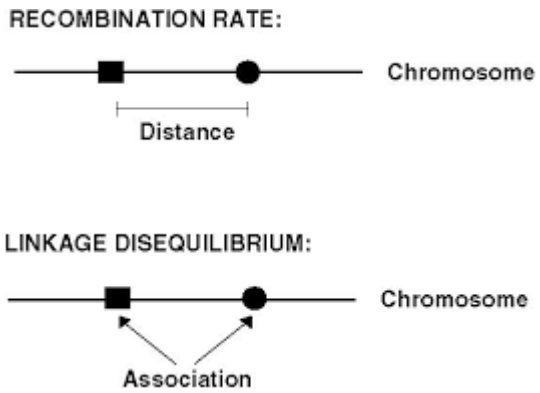
7/12

3/10

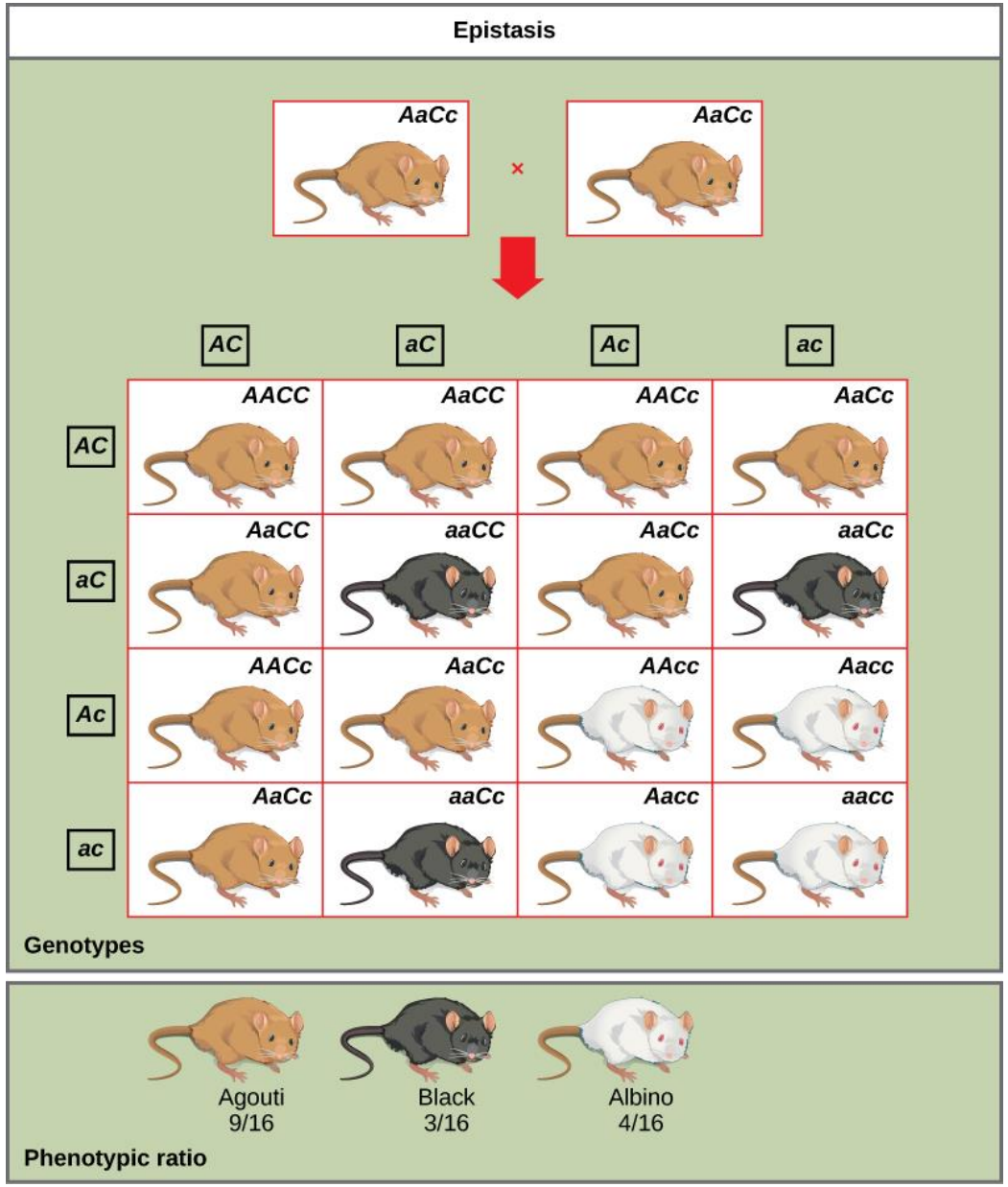
4/10

Gene-gene interaction

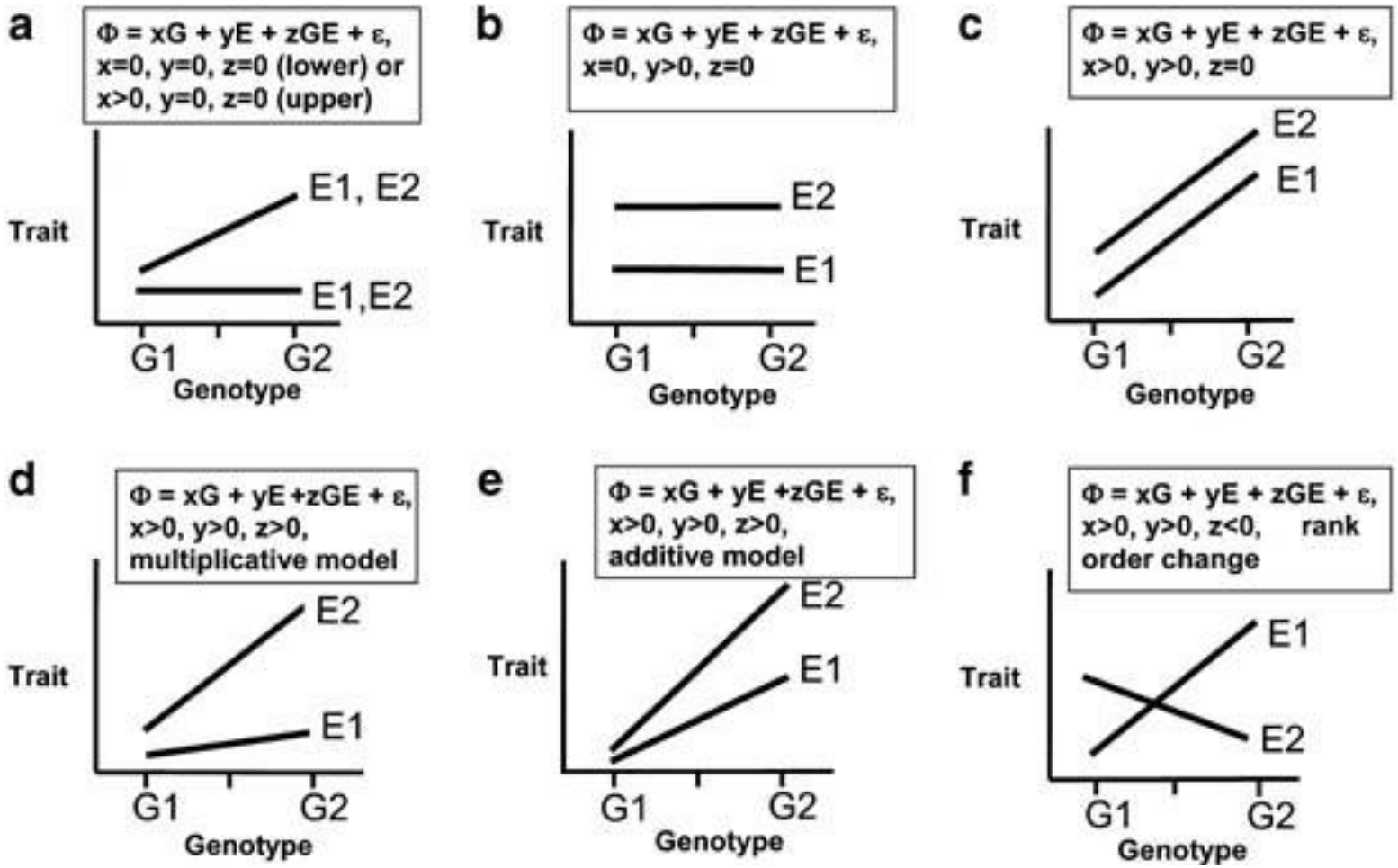
- Linkage disequilibrium



- Epistasis: non-addictive effect

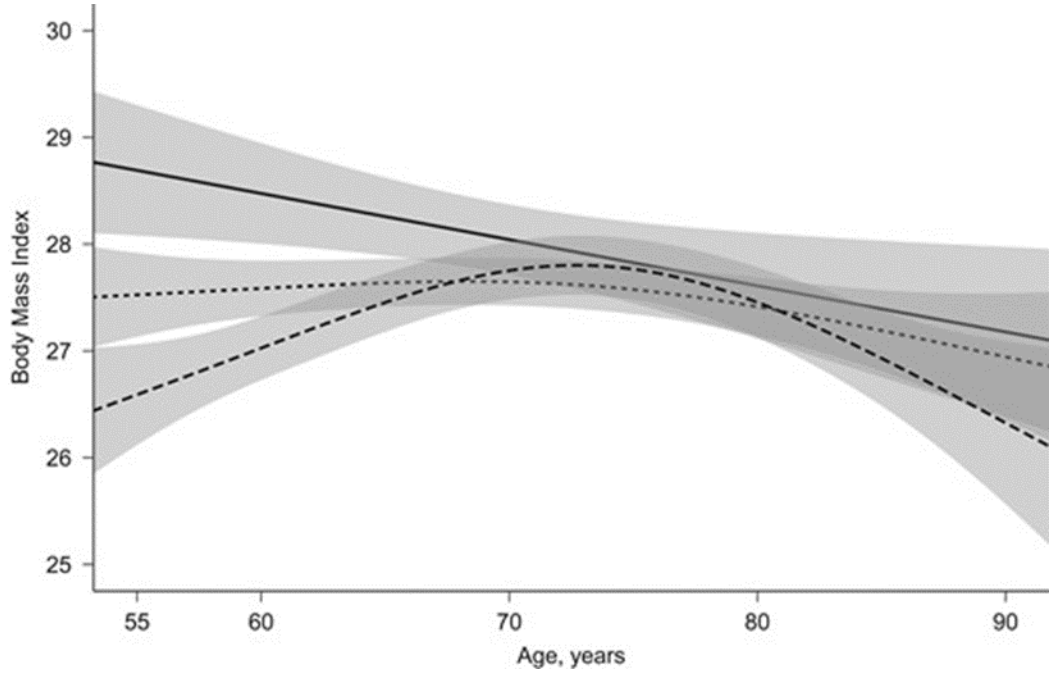


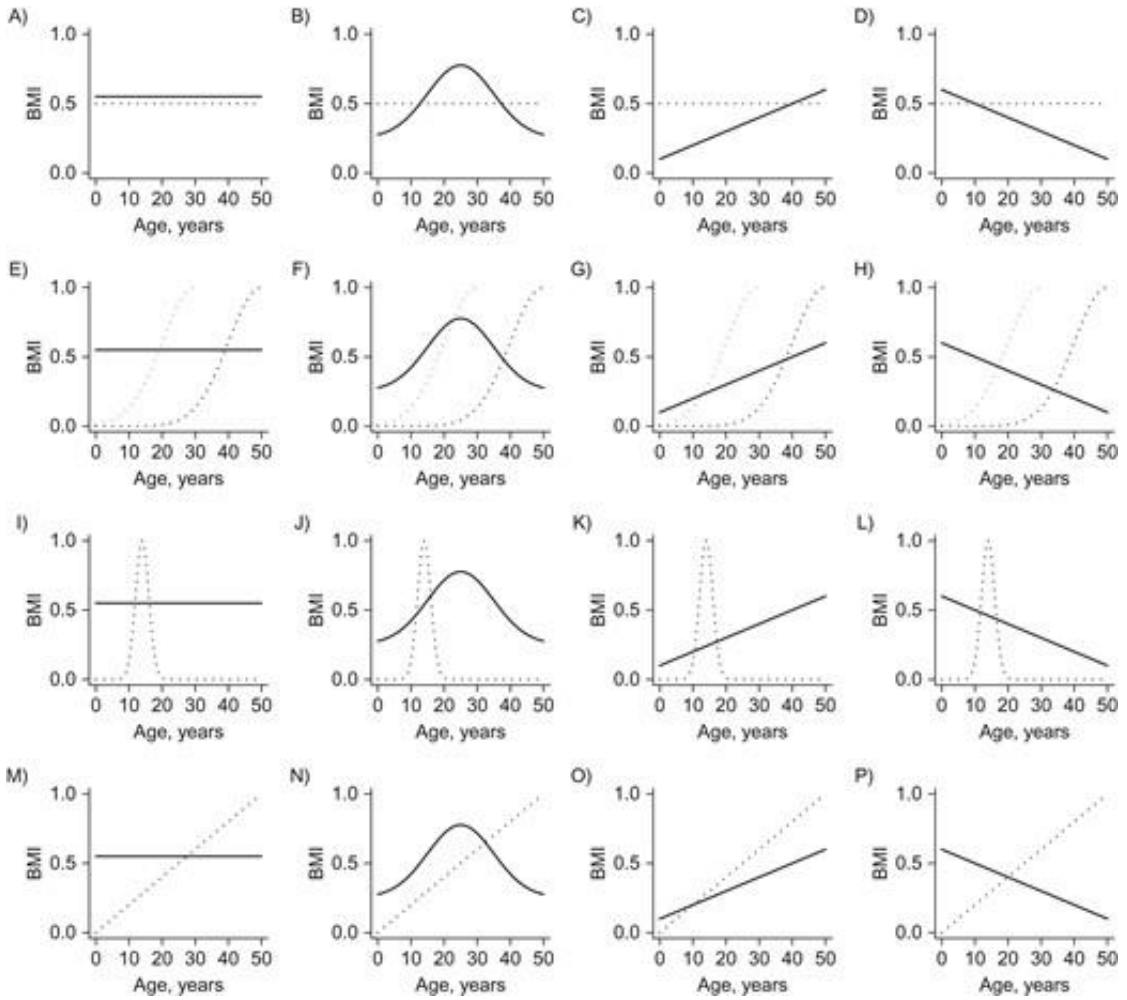
Gene-environment interaction



Time-varying exposure

canalization: the robustness of phenotypes to perturbation

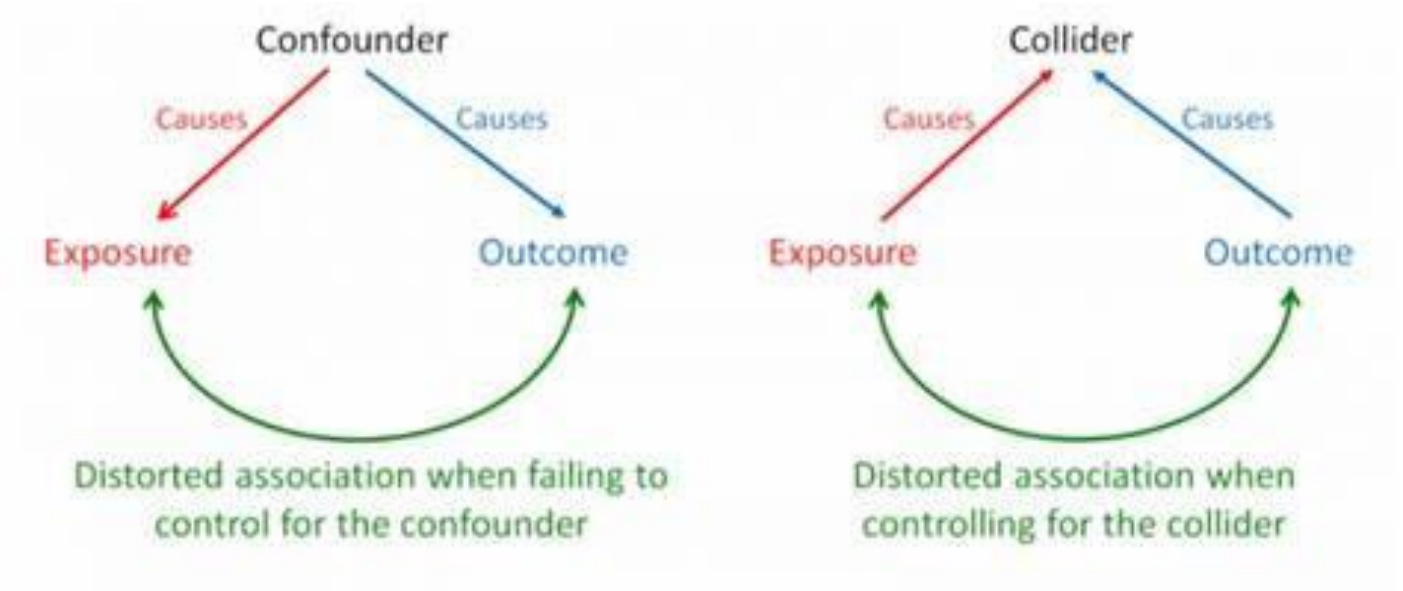




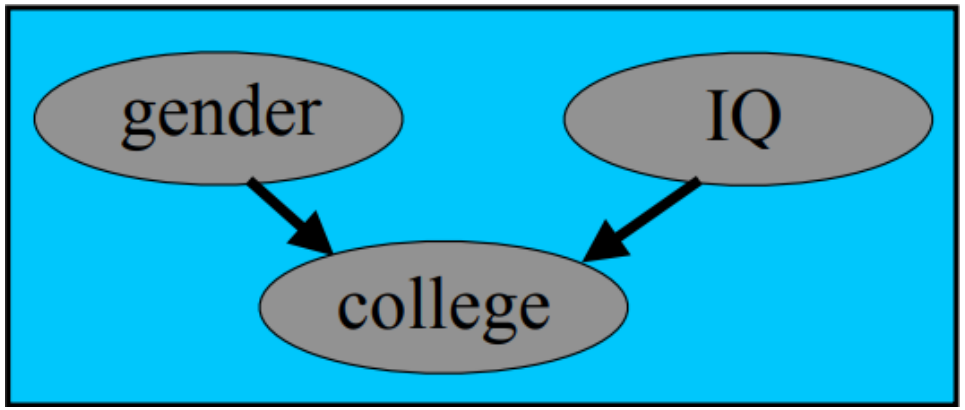
Reverse causality

- Bidirectional / reciprocal MR
- MR Steiger directionality test:
  - In MR it is assumed that the instruments influence the exposure first and then the outcome through the exposure.
  - IV解释exposure的能力应该比解释outcome的能力更强

Collider bias



Go back 50 years; in Western world, female college students were smarter than male ones on average. Why?



三角证据 triangulation

TRIANGULATION

A checklist.

- The different approaches address the same underlying question.
- The key sources of bias for each approach are explicitly acknowledged.
- For each approach, the expected directions of all key sources of potential bias are made explicit, where feasible.
- Ideally, some of the approaches being compared will have potential biases that are in opposite directions.
- Ideally, results from more than two approaches — which have different and unrelated key sources of potential biases — are compared. Source: ref. 3

COMMENT

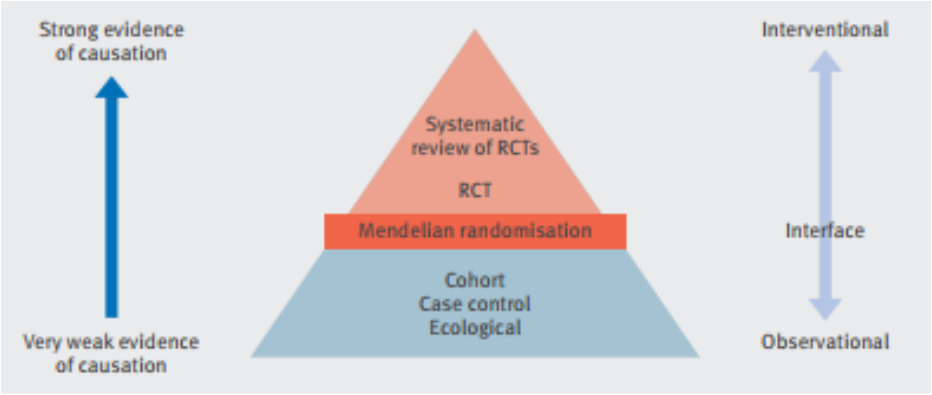
TECHNOLOGY From training to therapy — applications of virtual reality surveyed p.402 | CULTURE Biography of YouTube maps a parallel universe of viral video p.403 | PLASTICS China's ban on imported waste could boost sustainability p.405 | OBITUARY Calestous Juma, African science champion, remembered p.406

ILLUSTRATION BY DAVID PARKINS

Repeating experiments is not enough

如何开展MR研究

As readers



Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians

Neil M Davies,[1,2] Michael V Holmes,[1,3,4,5] George Davey Smith[1,2,6]

Box 2: Critical appraisal checklist for evaluating Mendelian randomisation studies

Some key questions readers can ask below.

Core Mendelian randomisation assumptions

- Is there sufficient evidence that the genetic variants are robustly associated with the risk factor of interest?
- Are the genetic variants associated with potential confounders? Do the authors present this relationship?
- Is there any way for the genetic variants to affect the outcome through alternative pathways (horizontal pleiotropy)? Do the authors present alternative Mendelian randomisation approaches (such as MR Egger, median, and mode estimators, or use of "negative control" populations) to investigate this more fully?

Methods reporting

All studies

- Are the effect and other alleles coded in the same direction for the exposure and outcome?

Two sample studies

- Were the two samples drawn from the same population?
- Were the two samples independent?
- Was the analysis restricted to independent variants (that is, pruned of SNPs in linkage disequilibrium) or did the analysis allow for the correlation between variants?

Data presentation

- Do the authors present the results as a genetic association, an instrumental variable estimate, or both?
- If they provide an instrumental variable estimate, do they compare it with the conventional observational estimate?
- Do the authors provide sensitivity analyses such as MR Egger, weighted median, and mode Mendelian randomisation, or use negative control populations?
- Do the authors manually pick and choose which SNPs go into the instrument to tackle pleiotropy? If so, is the approach and justification clear?
- Do the authors provide the data that they used (especially for Mendelian randomisation analyses conducted at the summary level) in a supplement to allow researchers to reproduce their findings?

Interpretation

- If the Mendelian randomisation estimate is similar to the observational estimate and provides evidence in support of a causal effect, could it be due to weak instrument bias in a single study or confounding through, for example, horizontal pleiotropy?
- If the Mendelian randomisation estimate differs from the observational estimate and provides little evidence of a causal effect, could this be due to weak instrument bias when using two different samples or negative confounding due to pleiotropy?
- Mendelian randomisation provides estimates of the effects of the risk factor over a lifetime, and the numerical effect estimates may not be clinically meaningful. Will interventions at a specific age have the same sized effects?
- Are the 95% confidence intervals of the Mendelian randomisation estimate sufficiently precise to identify the observational estimate and a clinically meaningful difference?

Clinical implications

- Do the results triangulate with other forms of evidence? Could a clinical trial be conducted to provide definitive evidence, as in the case of PCSK9 inhibitors?
- If a randomised clinical trial is not feasible (such as in the case of alcohol consumption and risk of heart disease) or unlikely to be conducted in the short term (such as the case of lifestyle interventions to lower BMI and risk of heart disease), and there is existing evidence from multiple Mendelian randomisation studies and other robust study designs that converge on a similar result and show consistency of association, this information can be used to guide patient care; for example, advising weight loss to prevent heart disease or advising against moderate alcohol consumption to prevent cardiovascular disease

How to conduct MR analysis

METHOD ARTICLE

UPDATE **Guidelines for performing Mendelian randomization investigations: update for summer 2023 [version 3; peer review: 2 approved]**

Stephen Burgess[1,2], George Davey Smith[3,4], Neil M. Davies[3,5-7], Frank Dudbridge[8], Dipender Gill[9], M. Maria Glymour[10], Fernando P. Hartwig[3,11], Zoltán Kutalik[12-14], Michael V. Holmes[15,16], Cosetta Minelli[17], Jean V. Morrison[18], Wei Pan[19], Caroline L. Relton[3,4,20], Evropi Theodoratou[21,22]

1. If there are genetic variants having biological relevance to the exposure, then consider performing the MR analysis using these variants only, and perform appropriate sensitivity analyses.

2. If such variants are not available, consider initially performing a "liberal" MR analysis using a less stringent choice of variants. If the estimate is null, then there is little evidence for a causal effect.

3. If the estimate from the initial analysis is non-null, then assess the robustness of the finding using different approaches: stricter criteria for variant selection, leave-one-out analyses, robust methods, positive/negative controls, subgroup analyses, colocalization (for analyses based on single gene region).

What is the aim of the Mendelian randomization investigation?

To assess the causal role of an exposure
Priorities should be:
- validity of the instrumental variable assumptions
- precision and relevance of the gene—outcome associations

To evaluate the quantitative impact of an intervention on the exposure
In addition to the above, extra priorities should be:
- how well the genetic variant proxies the intervention
- whether genetic analyses are conducted in a relevant population,
- linearity and homogeneity of relationships between variables

Note: estimate typically represents impact of lifelong change in the exposure

Should I perform a one- or a two-sample investigation?

One-sample		Two-sample	
Advantages:	Concerns:	Advantages:	Concerns:
- Harmonization	- Weak instrument bias	- Power	- Similarity of samples
- Subgroup analyses		- Transparency	
- BUT difficult to find single relevant sample		- Easier practically	

How to select genetic variants?
What sensitivity and supplementary analyses should I perform?

If there are genetic variants having biological relevance to the exposure...
... then consider performing an MR analysis using these variants only.
Advantages:
- Instrumental variable assumptions more plausible
- Relevance to intervention often more clear

Concerns:
- Low power
- Results sensitive if locus is pleiotropic

Sensitivity analyses:
- Single locus: colocalization. Multiple loci: assess heterogeneity
- Consider positive and negative control outcomes

If such variants are not available...
... then consider performing an agnostic polygenic MR analysis.

Advantages:	Concerns:
- Can use robust methods	- Pleiotropy is likely

Sensitivity analyses:
- Assess heterogeneity: statistical test and graphically (e.g. scatter plot)
- Perform a range of robust methods making different assumptions
- Check genetic associations with variables on pleiotropic pathways
- Liberal and conservative choices of variants, leave-one-out analyses
- Conduct relevant subgroup analysis

Database resources

Table 3 | Databases of genome-wide association study results

Data source	Description	Number of traits	Integrated with statistics package?
MR-Base	A curated database of genome-wide association study results with integrated R package for MR[23]	Over 1000	Yes
PhenoScanner	A curated database of genome-wide association study results with integrated R package for MR[37]	Over 500	Yes
GWAS catalog	Searchable database of genome-wide association study results[38]	Over 24 000	No

Table 2 | Publicly available data sources for two sample Mendelian randomisation studies

Consortium name	Description	Most recent sample size
BCAC[24]	Breast cancer	256 123
CARDIoGRAMplusC4D[25]	Coronary artery disease and myocardial infarction	184 305
CKDGen[26]	Chronic kidney disease	111 666
DIAGRAM[27]	Diabetes	159 208
EAGLE[28]	Antenatal and early life and childhood phenotypes	47 541
EGG[29]	Early growth	153 781
GIANT[30]	Height, BMI, and other adiposity traits	693 529
GLGC[31]	Global lipids genetics consortium	331 368
ISGC[32]	Stroke	84 961
MAGIC[33]	Glucose and insulin related traits	224 459
PGC[34 35]	Psychiatric genetics, alcohol and tobacco, and other related traits	>500 000
SSGAC[36]	Educational attainment and wellbeing	293 723

STROBE-MR

Clinical Review & Education

JAMA | Special Communication

Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization
The STROBE-MR Statement

Veronika W. Skrivankova, PhD; Rebecca C. Richmond, PhD; Benjamin A. R. Woolf, MSc; James Yarmolinsky, PhD; Neil M. Davies, PhD; Sonja A. Swanson, ScD; Tyler J. VanderWeele, PhD; Julian P. T. Higgins, PhD; Nicholas J. Timpson, PhD; Niki Dimou, PhD; Claudia Langenberg, PhD; Robert M. Golub, MD; Elizabeth W. Loder, MD; Valentina Gallo, PhD; Anne Tybjaerg-Hansen, MD, DMSc; George Davey Smith, MD, DSc; Matthias Egger, MD; J. Brent Richards, MD

STROBE-MR

Item No.	Section	Checklist item
Title and Abstract		
1	Title and abstract	Indicate mendelian randomization (MR) as the study's design in the title and/or the abstract if that is a main purpose of the study.
Introduction		
2	Background	Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question.
3	Objectives	State specific objectives clearly, including prespecified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects.
Methods		
4	Study design and data sources	Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:
	a	Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available.
	b	Participants: Report the eligibility criteria and the sources and methods of selection of participants. Report the sample size and whether any power or sample size calculations were carried out prior to the main analysis.
	c	Describe measurement, quality control, and selection of genetic variants.
	d	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases.
	e	Provide details of ethics committee approval and participant informed consent, if relevant.
5	Assumptions	Explicitly state the 3 core instrumental variable (IV) assumptions for the main analysis (relevance, independence, and exclusion restriction), as well assumptions for any additional or sensitivity analysis.
6	Statistical methods: main analysis	Describe statistical methods and statistics used.
	a	Describe how quantitative variables were handled in the analyses (ie, scale, units, model).
	b	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected.
	c	Describe the MR estimator (eg, 2-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of 2-sample MR, whether the same covariate set was used for adjustment in the 2 samples.
	d	Explain how missing data were addressed.
	e	If applicable, indicate how multiple testing was addressed.

STROBE-MR

7	Assessment of assumptions	Describe any methods or prior knowledge used to assess the assumptions or justify their validity.
8	Sensitivity analyses and additional analyses	Describe any sensitivity analyses or additional analyses performed (eg, comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations).
9	Software and preregistration	
	a	Name statistical software and package(s), including version and settings used.
	b	State whether the study protocol and details were preregistered (as well as when and where).
Results		
10	Descriptive data	
	a	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram.
	b	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (eg, means, SDs, proportions).
	c	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies.
	d	For 2-sample MR: i. Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples. ii. Provide information on the number of individuals who overlap between the exposure and outcome studies.
11	Main results	
	a	Report the associations between genetic variant and exposure and between genetic variant and outcome, preferably on an interpretable scale.
	b	Report MR estimates of the relationship between exposure and outcome and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference.
	c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.
	d	Consider plots to visualize results (eg, forest plot, scatterplot of associations between genetic variants and outcome vs between genetic variants and exposure).
12	Assessment of assumptions	
	a	Report the assessment of the validity of the assumptions.
	b	Report any additional statistics (eg, assessments of heterogeneity across genetic variants, such as I^2, Q statistic, or E-value).

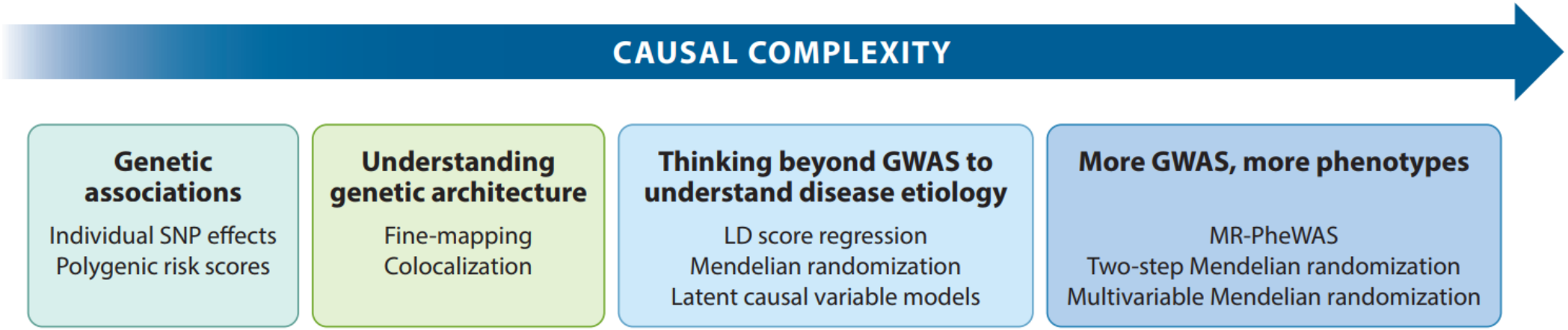
STROBE-MR

Item No.	Section	Checklist item
13	Sensitivity analyses and additional analyses	
	a	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions.
	b	Report results from other sensitivity analyses or additional analyses.
	c	Report any assessment of the direction of the causal relationship (eg, bidirectional MR).
	d	When relevant, report and compare with estimates from non-MR analyses.
	e	Consider additional plots to visualize results (eg, leave-one-out analyses).
Discussion		
14	Key results	Summarize key results with reference to study objectives.
15	Limitations	Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them.
16	Interpretation	
	a	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies.
	b	Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions.
	c	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions.
17	Generalizability	Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure.
Other Information		
18	Funding	Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based.
19	Data and data sharing	Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article or report whether the code is publicly accessible and, if so, where.
20	Conflicts of interest	All authors should declare all potential conflicts of interest.

报告规范

论文部分/主题		编号	内容
标题和摘要		1	在标题及摘要中说明研究目的、研究设计及主要的暴露与结局变量；在摘要中对研究方法及所得结论做简要总结。
前言		2	阐述研究背景及 MR 方法的基本原理；阐明研究目的，提出研究假设。
方法			
	研究设计	3	阐述真实世界研究设计。
	资料来源	4	说明研究样本选择的纳入及排除标准。
	研究变量	5	说明研究的暴露及结局变量。
	工具变量选择	6	阐明筛选工具变量的标准及具体步骤。
	工具变量强度检验	7	对纳入模型中的工具变量强度进行估计与评价，避免弱工具变量偏倚的产生。
	统计效能估计	8	根据样本含量、工具变量解释暴露变异比例等信息对模型的统计效能进行预测。
	多效性偏倚的校正	9	检验模型中是否存在多效性工具变量；存在多效性工具变量情况下，说明采用何种方式对潜在的多效性偏倚加以校正。
	统计模型的构建	10	描述拟采用的模型及模型构建思想。当样本存在异质性时，需采用相应的措施（分层分析，主成分分析）加以修正，避免人群分层所导致的偏倚；当工具变量间存在连锁不平衡时，需将工具变量之间的相关性信息纳入模型；当存在多效性工具变量时，需构建基于校正多效性偏倚的模型。
结果			
	研究对象	11	描述选定样本的基本人口学特征，明确各个子样本是否同质。
	数据质量评价	12	描述真实世界数据的缺失情况，异常值情况等。
	工具变量选择	13	描述选定工具变量的个数、工具变量之间的相关性结构以及工具变量解释暴露变异的比例等内容，给出工具变量强度及模型统计效能的估计结果。
	主要结果	14	报告经过校正工具变量多效性效应、连锁不平衡等问题后由 MR 模型得到的结果。
	其他分析	15	报告进行的其他分析结果，如敏感性分析，针对不同性别、年龄等因素进行的分层分析等结果。
讨论			
	重要结论	16	根据研究内容以及研究结果总结关键结论。
	结果解释	17	结合研究结果，已有的相关研究结论及生物学机制等先验背景知识，对本研究所得到的结果给出综合的、合理的解释。
	局限性	18	客观讨论研究的局限性，包括应用模型的局限性，因果效应结论的外推性等。
其他信息			
	资源	19	给出样本数据获得渠道、统计分析应用软件等相关信息及链接。
	伦理	20	说明研究内容、设计及样本数据收集等过程是否符合伦理要求。

More analysis



案例分析

nature neuroscience

ARTICLES

https://doi.org/10.1038/s41593-018-0326-7

Genome-wide meta-analysis of depression identifies 102 independent variants and highlights the importance of the prefrontal brain regions

ARTICLES

https://doi.org/10.1038/s41588-018-0311-9

nature genetics

Corrected: Author Correction

Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk

nature genetics

Article

https://doi.org/10.1038/s41588-022-01286-7

Genome-wide meta-analysis identifies 93 risk loci and enables risk prediction equivalent to monogenic forms of venous thromboembolism

Thanks for listening!