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Sensitivity analyses in Mendelian randomization studies

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(Some slides adapted from Prof David Evans)

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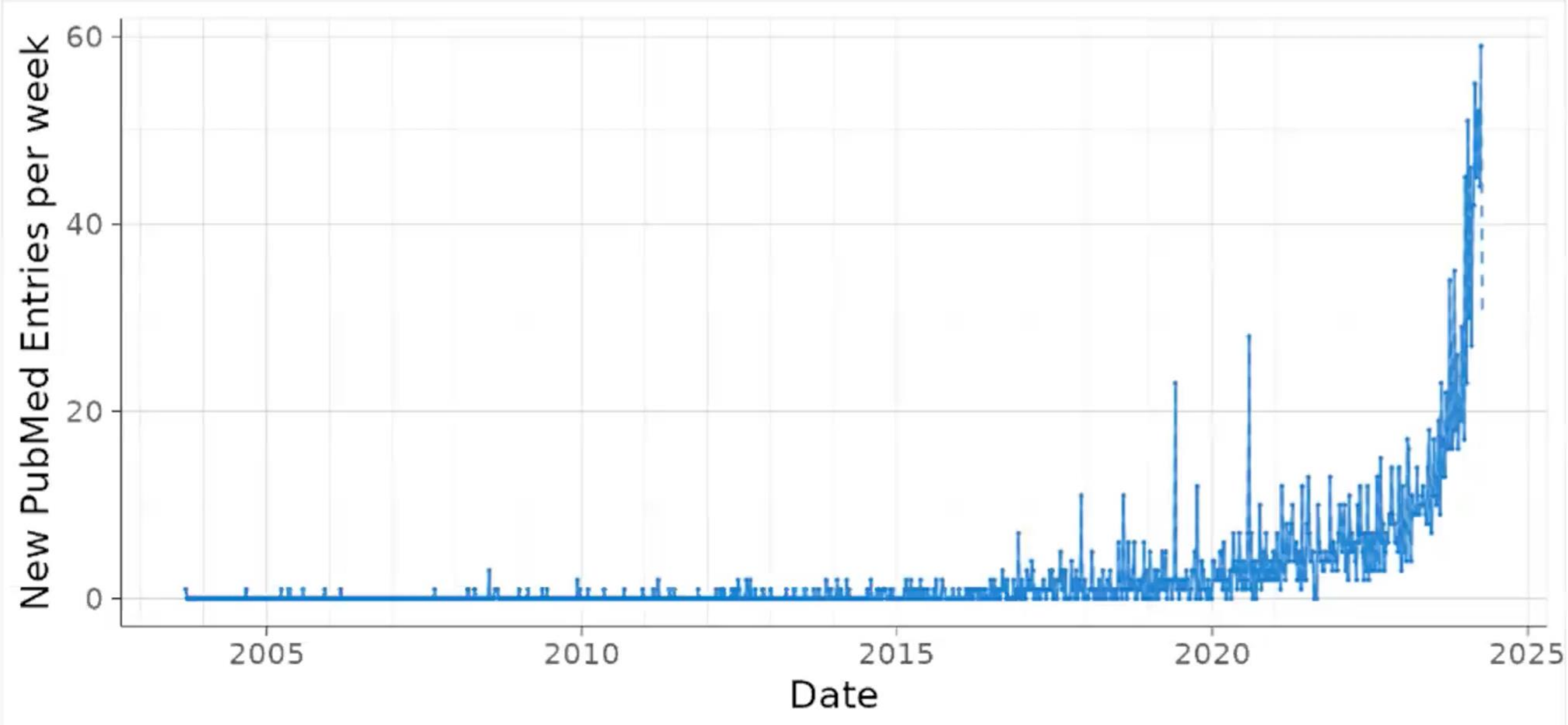
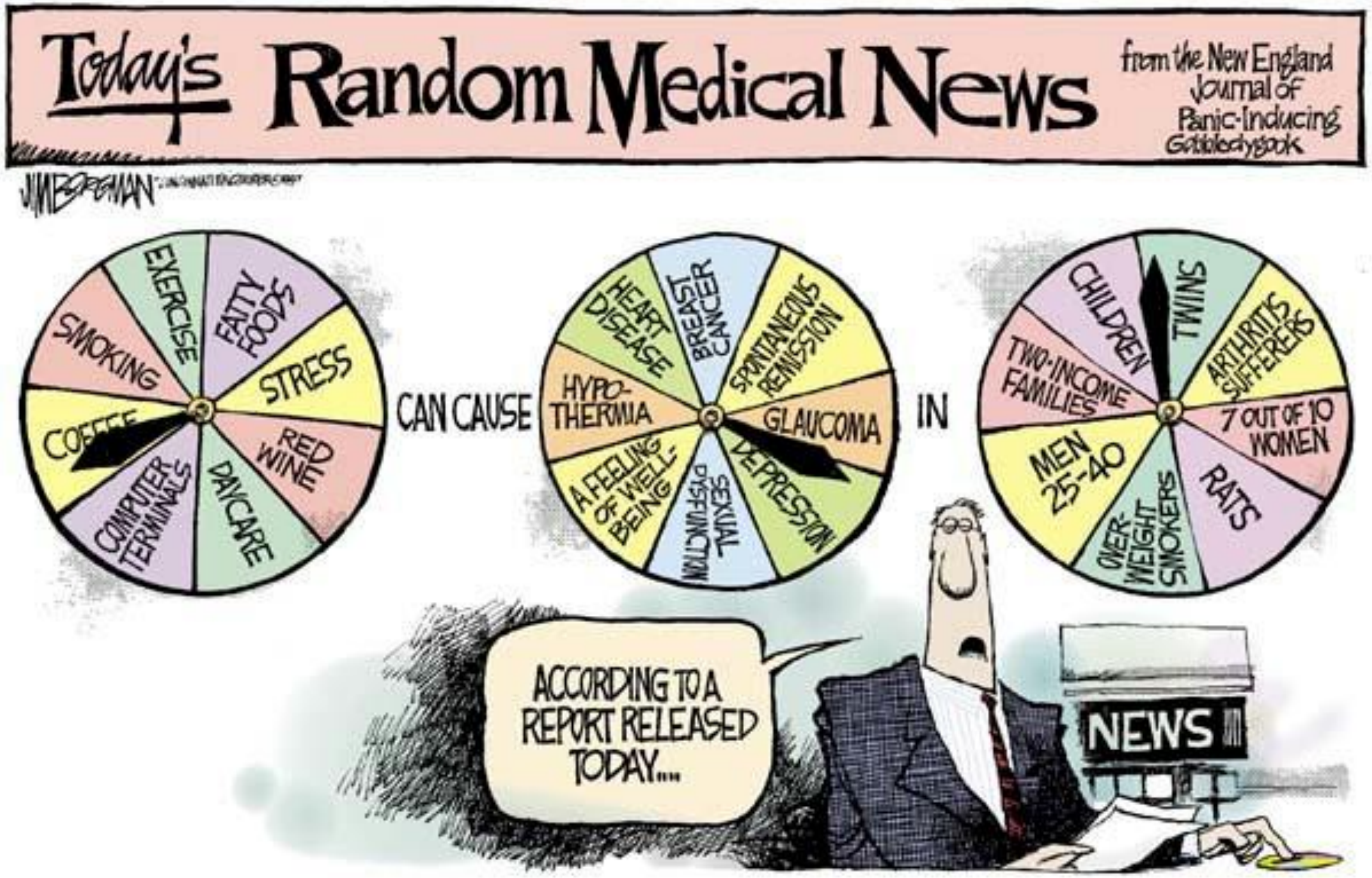
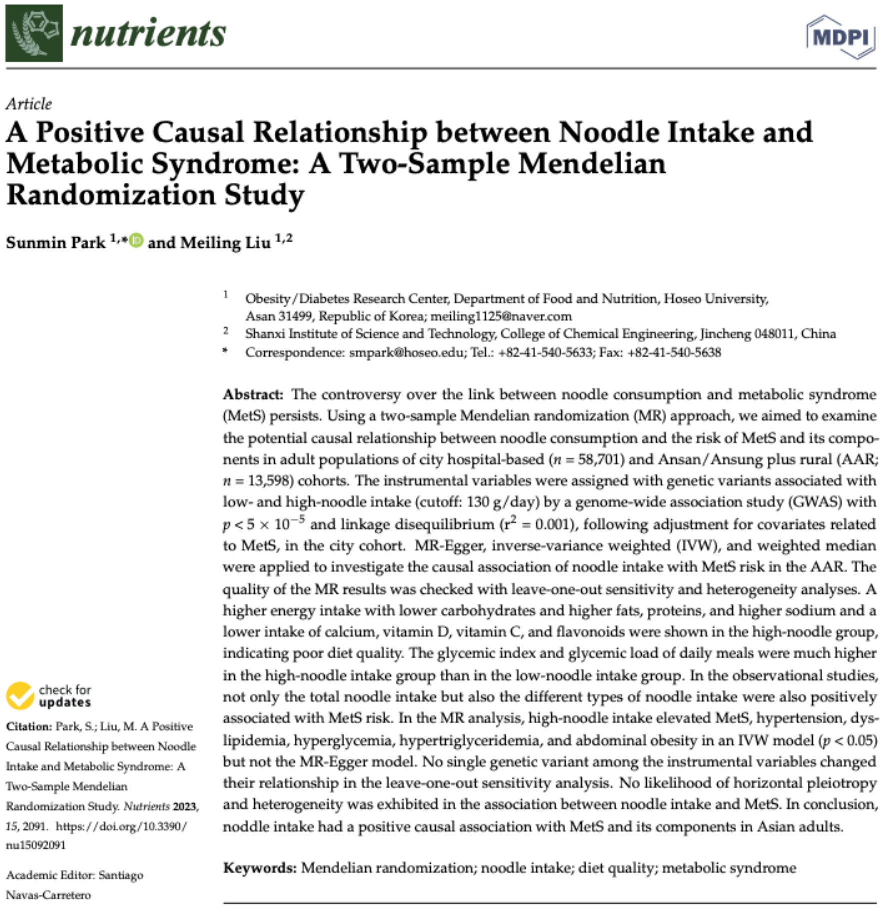
PubMed search for Mendelian randomi[z/s]ation (title only)



Up to 2024-04-13

Problems with inferring causality in ~~observational~~ MR studies





nutrients MDPI

Article

A Positive Causal Relationship between Noodle Intake and Metabolic Syndrome: A Two-Sample Mendelian Randomization Study

Sunmin Park [1,*] **and Meiling Liu** [1,2]

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Abstract: The controversy over the link between noodle consumption and metabolic syndrome (MetS) persists. Using a two-sample Mendelian randomization (MR) approach, we aimed to examine the potential causal relationship between noodle consumption and the risk of MetS and its components in adult populations of city hospital-based (*n* = 58,701) and Ansan/Ansung plus rural (AAR; *n* = 13,598) cohorts. The instrumental variables were assigned with genetic variants associated with low- and high-noodle intake (cutoff: 130 g/day) by a genome-wide association study (GWAS) with $p < 5 \times 10^{-5}$ and linkage disequilibrium ($r^2 = 0.001$), following adjustment for covariates related to MetS, in the city cohort. MR-Egger, inverse-variance weighted (IVW), and weighted median were applied to investigate the causal association of noodle intake with MetS risk in the AAR. The quality of the MR results was checked with leave-one-out sensitivity and heterogeneity analyses. A higher energy intake with lower carbohydrates and higher fats, proteins, and higher sodium and a lower intake of calcium, vitamin D, vitamin C, and flavonoids were shown in the high-noodle group, indicating poor diet quality. The glycemic index and glycemic load of daily meals were much higher in the high-noodle intake group than in the low-noodle intake group. In the observational studies, not only the total noodle intake but also the different types of noodle intake were also positively associated with MetS risk. In the MR analysis, high-noodle intake elevated MetS, hypertension, dyslipidemia, hyperglycemia, hypertriglyceridemia, and abdominal obesity in an IVW model ($p < 0.05$) but not the MR-Egger model. No single genetic variant among the instrumental variables changed their relationship in the leave-one-out sensitivity analysis. No likelihood of horizontal pleiotropy and heterogeneity was exhibited in the association between noodle intake and MetS. In conclusion, noddle intake had a positive causal association with MetS and its components in Asian adults.

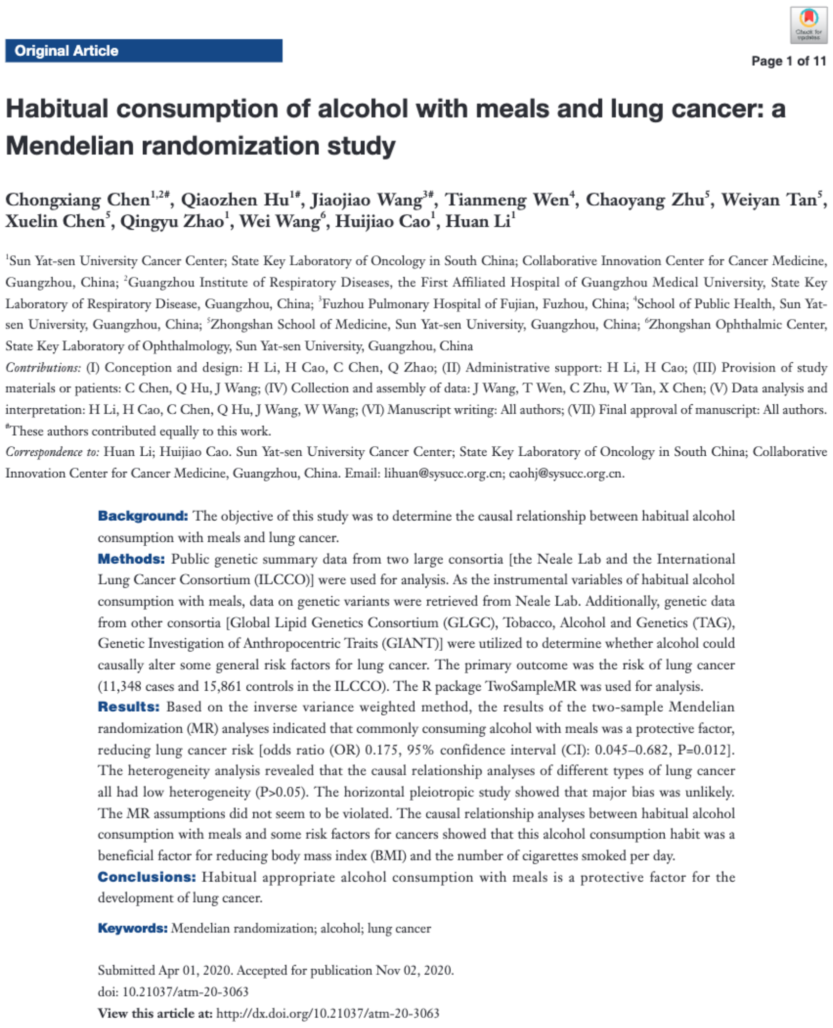
Keywords: Mendelian randomization; noodle intake; diet quality; metabolic syndrome

check for updates

Citation: Park, S.; Liu, M. A Positive Causal Relationship between Noodle Intake and Metabolic Syndrome: A Two-Sample Mendelian Randomization Study. *Nutrients* **2023**, *15*, 2091. https://doi.org/10.3390/nu15092091

Academic Editor: Santiago Navas-Carretero

High noodle intake (>130g/day) is causally associated with a higher risk of metabolic syndrome (OR: 1.196 [1.045,1.368]; p = 0.0009).



Original Article

Page 1 of 11

Habitual consumption of alcohol with meals and lung cancer: a Mendelian randomization study

Chongxiang Chen[1,2#], **Qiaozhen Hu**[1#], **Jiaojiao Wang**[3#], **Tianmeng Wen**[4], **Chaoyang Zhu**[5], **Weiyan Tan**[5], **Xuelin Chen**[5], **Qingyu Zhao**[1], **Wei Wang**[6], **Huijiao Cao**[1], **Huan Li**[1]

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Contributions: (I) Conception and design: H Li, H Cao, C Chen, Q Zhao; (II) Administrative support: H Li, H Cao; (III) Provision of study materials or patients: C Chen, Q Hu, J Wang; (IV) Collection and assembly of data: J Wang, T Wen, C Zhu, W Tan, X Chen; (V) Data analysis and interpretation: H Li, H Cao, C Chen, Q Hu, J Wang, W Wang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Huan Li; Huijiao Cao. Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center for Cancer Medicine, Guangzhou, China. Email: lihuan@sysucc.org.cn; caohj@sysucc.org.cn.

Background: The objective of this study was to determine the causal relationship between habitual alcohol consumption with meals and lung cancer.

Methods: Public genetic summary data from two large consortia [the Neale Lab and the International Lung Cancer Consortium (ILCCO)] were used for analysis. As the instrumental variables of habitual alcohol consumption with meals, data on genetic variants were retrieved from Neale Lab. Additionally, genetic data from other consortia [Global Lipid Genetics Consortium (GLGC), Tobacco, Alcohol and Genetics (TAG), Genetic Investigation of Anthropocentric Traits (GIANT)] were utilized to determine whether alcohol could causally alter some general risk factors for lung cancer. The primary outcome was the risk of lung cancer (11,348 cases and 15,861 controls in the ILCCO). The R package TwoSampleMR was used for analysis.

Results: Based on the inverse variance weighted method, the results of the two-sample Mendelian randomization (MR) analyses indicated that commonly consuming alcohol with meals was a protective factor, reducing lung cancer risk [odds ratio (OR) 0.175, 95% confidence interval (CI): 0.045–0.682, P=0.012]. The heterogeneity analysis revealed that the causal relationship analyses of different types of lung cancer all had low heterogeneity (P>0.05). The horizontal pleiotropic study showed that major bias was unlikely. The MR assumptions did not seem to be violated. The causal relationship analyses between habitual alcohol consumption with meals and some risk factors for cancers showed that this alcohol consumption habit was a beneficial factor for reducing body mass index (BMI) and the number of cigarettes smoked per day.

Conclusions: Habitual appropriate alcohol consumption with meals is a protective factor for the development of lung cancer.

Keywords: Mendelian randomization; alcohol; lung cancer

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View this article at: http://dx.doi.org/10.21037/atm-20-3063

Habitual light-to-moderate alcohol consumption with meals reduced risk of lung cancer (OR: 0.175 [0.045-0.682]; p=0.012) and lung squamous cell cancer (OR: 0.075 [0.013-0.429]; p=0.004).

Recap

- Mendelian randomization is a technique that uses genetically informative observational data to inform causality.

- Three core assumptions:

(1) Relevance assumption: SNP is associated with the exposure

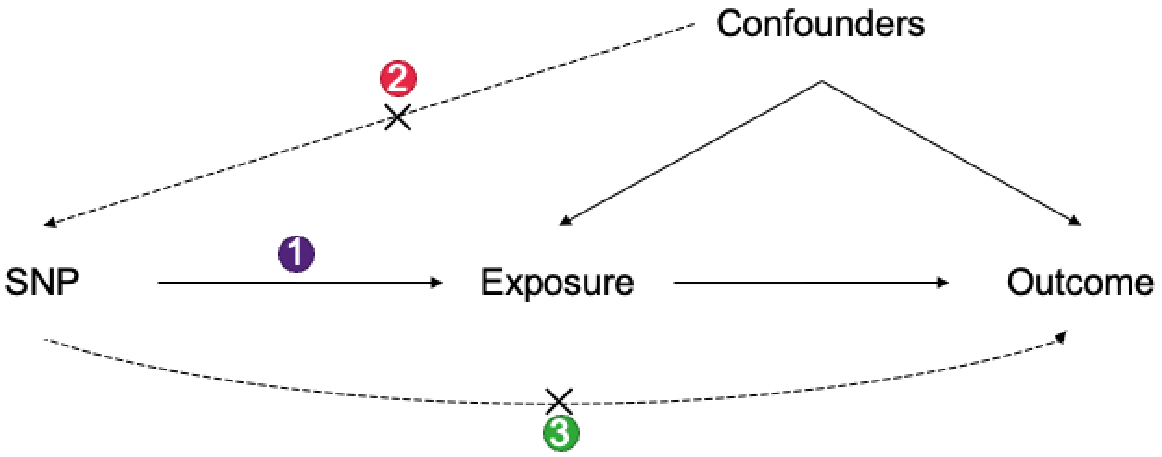
(2) Independence assumption: There are no confounding associations between the instrumental variables (IVs) and the outcome.

(3) Exclusion restriction: SNP ONLY associated outcome through the exposure

- Pleiotropy: Genetic variant influences more than one trait

- One-sample MR is where the SNP, exposure and outcome are all available in the same study.
- Two-sample MR is where the SNP-exposure association is measured in one study and the SNP outcome association is measured in a second study.

Recap
Assumption 1: Relevance assumption



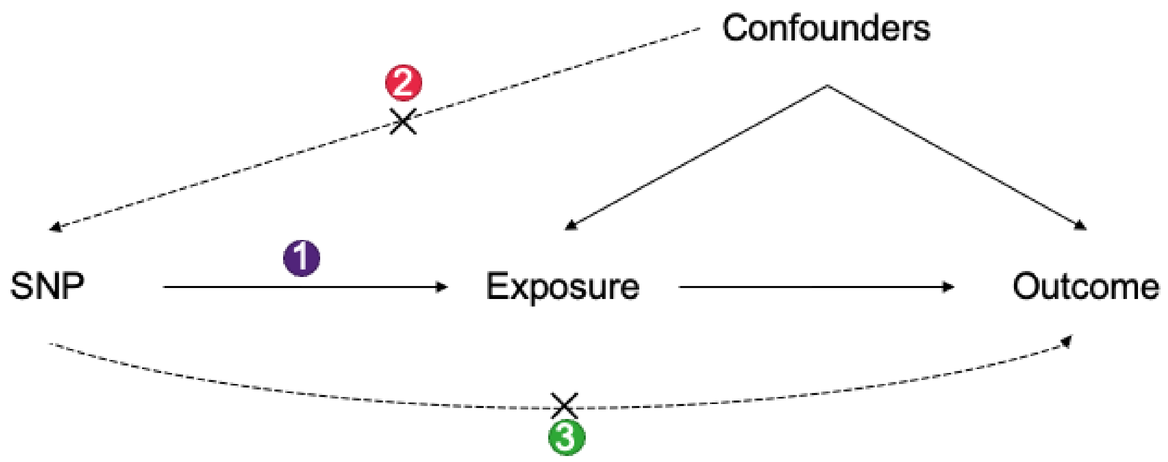
- Weak instruments:
  - Loss of power
  - Bias due to violations of the other assumptions will be amplified
- Bias towards outcome-risk factor association in one-sample MR or towards the null in two-sample MR – precision is also underestimated.
- Weak instruments can be detected using an F-statistic in one-sample MR (F-statistic > 10)

$$F_{\text{first}} = \frac{R^2 * (N-1)}{(1-R^2)}$$

Typically, SNPs which pass genome-wide significance ($P < 5 \times 10^{-8}$) and have been replicated in independent samples are used as IV's

Burgess S et al. *Int J Epidemiol.* (2011) 40(3):755-7645
Burgess S and Thompson SG. *Stat Med* (2011) 30(11):1312-1323

Recap
Assumption 2: Independence assumption



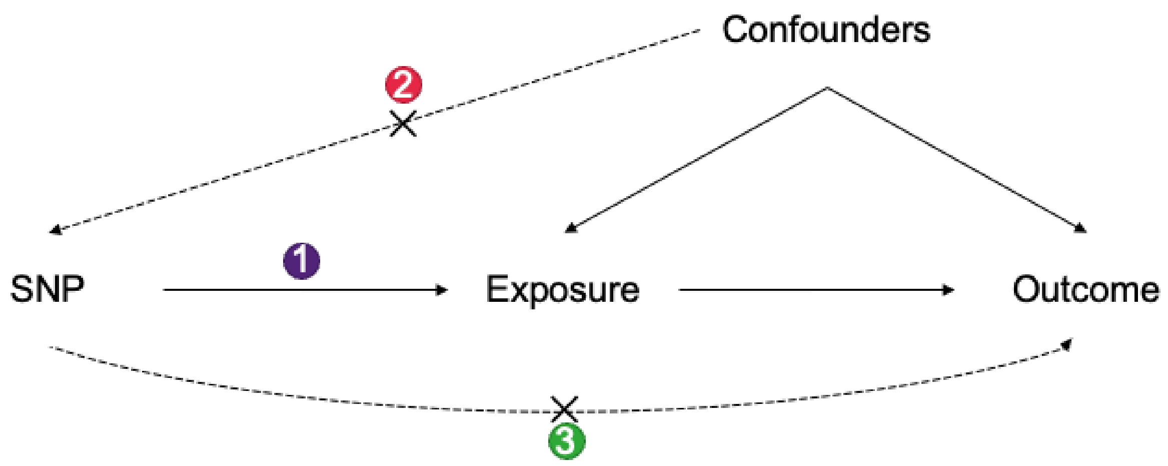
- Technically impossible to prove this assumption holds as we can’t test for association with *unobserved* confounders (need to rely on good knowledge of the science)

- May be possible to disprove by checking that the genetic variant is independent of *measured* confounders of the exposure-outcome relationship

- Factors that could influence the genetic variants and outcome include population stratification or structure, intergenerational (dynastic) effects and assortative mating.

Carter & Anderson (2024) *International Journal of Epidemiology*

Recap
Assumption 3: Exclusion restriction



- Again, is difficult to prove this assumption holds. Horizontal pleiotropy = SNP is associated with multiple traits independently of the exposure of interest

- Extensions to the basic MR design can be used to detect horizontal pleiotropy and estimate causal effect in its presence

Outline

1. Inverse variance weighted MR
2. Palindromic SNP / Ambiguous SNP

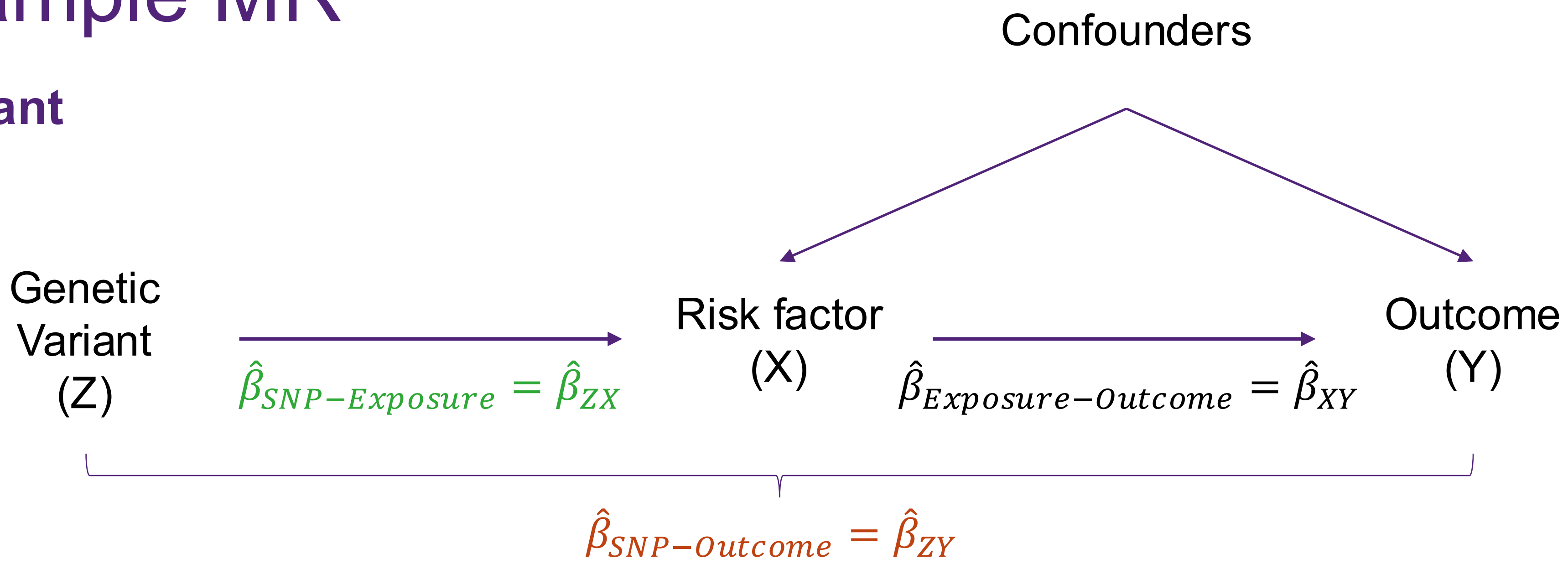
☕Mini-break

3. Methods to detect/account for horizontal pleiotropy
   - Heterogeneity tests
   - Multivariable MR
   - MR Egger
   - Median Estimator
   - Steiger Filtering

☕Afternoon tea

Two-sample MR

Single variant



Confounders

Genetic Variant (Z)

Risk factor (X)

Outcome (Y)

$\hat{\beta}_{SNP-Exposure} = \hat{\beta}_{ZX}$

$\hat{\beta}_{Exposure-Outcome} = \hat{\beta}_{XY}$

$\hat{\beta}_{SNP-Outcome} = \hat{\beta}_{ZY}$

Causal effect ($\hat{\beta}_{XY}$) by Wald estimator: $\dfrac{\hat{\beta}_{SNP-Outcome}}{\hat{\beta}_{SNP-Exposure}}$

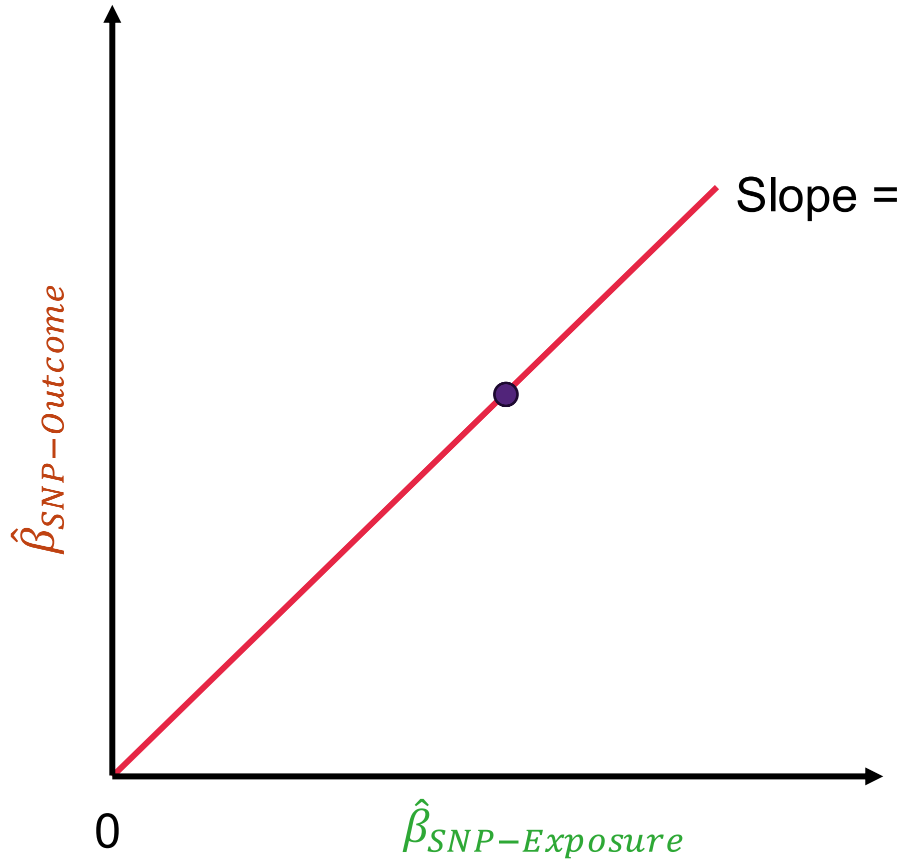
Standard error ($\hat{\sigma}_{XY}$) by Delta method: $\dfrac{\sigma_{SNP-Outcome}}{\hat{\beta}_{SNP-Exposure}}$

$\hat{\beta}_{SNP-Outcome} = \hat{\beta}_{SNP-Exposure}$ x $\hat{\beta}_{Exposure-Outcome}$

Can be estimated in different samples (e.g. two-sample MR)

Two-sample MR

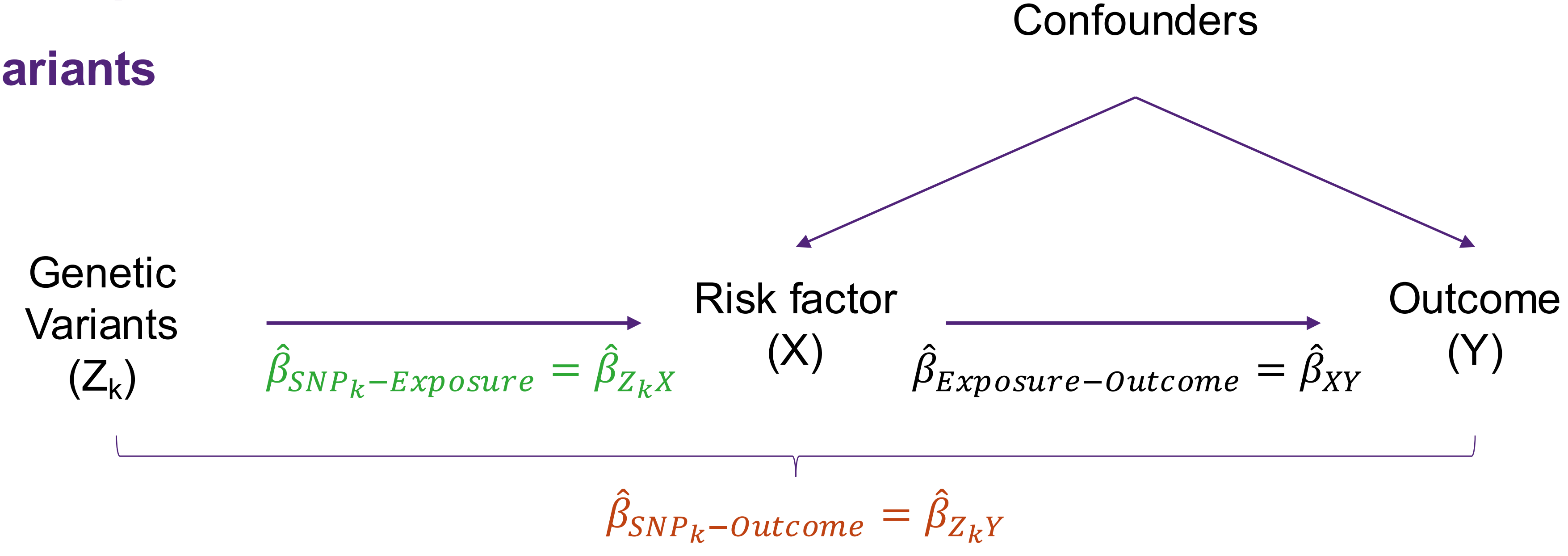
Single variant



Slope = Causal effect ($\hat{\beta}_{XY}$) = $\frac{\hat{\beta}_{SNP-Outcome}}{\hat{\beta}_{SNP-Exposure}}$

Two-sample MR

Multiple variants



Confounders

Genetic Variants (Z_k)

$\hat{\beta}_{SNP_k-Exposure} = \hat{\beta}_{Z_kX}$

Risk factor (X)

$\hat{\beta}_{Exposure-Outcome} = \hat{\beta}_{XY}$

Outcome (Y)

$\hat{\beta}_{SNP_k-Outcome} = \hat{\beta}_{Z_kY}$

Causal effect by Wald estimator:

$$\hat{\beta}_{XY_k} = \frac{\hat{\beta}_{SNP_k-Outcome}}{\hat{\beta}_{SNP_k-Exposure}}$$

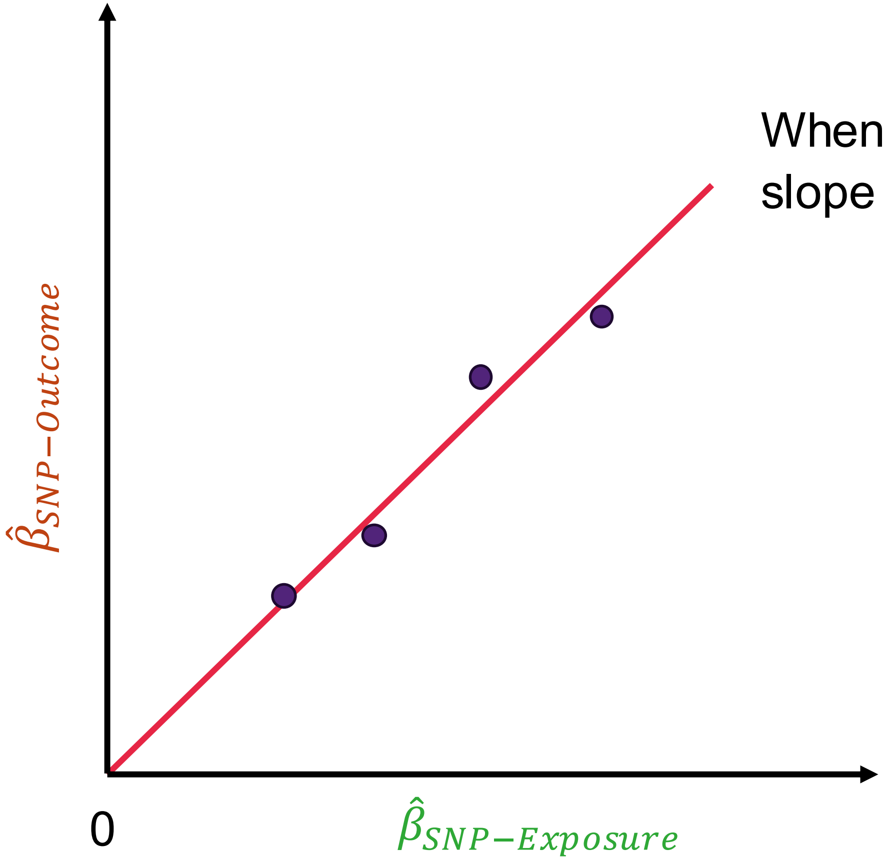
Inverse variance weighted (IVW) average causal effect:

$$\hat{\beta}_{IVW} = \frac{\sum_{k=1}^{K} \hat{\beta}_{XY_k} w_k}{\sum_{k=1}^{K} w_k}$$

Where $w_k = \frac{1}{var(\hat{\beta}_{XY_k})} = \frac{1}{\hat{\sigma}_{XY_k}^{2}}$ is the inverse variance of the causal effect estimated from the kth genetic variant

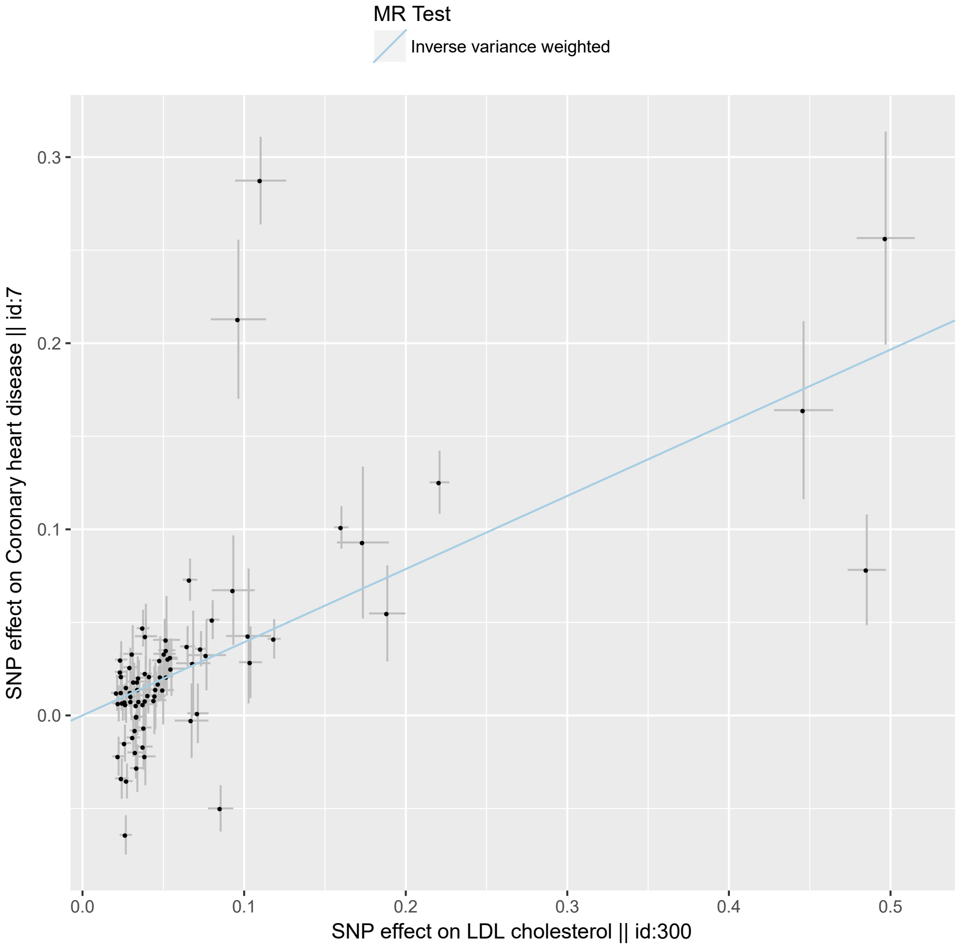
Two-sample MR

Multiple variants

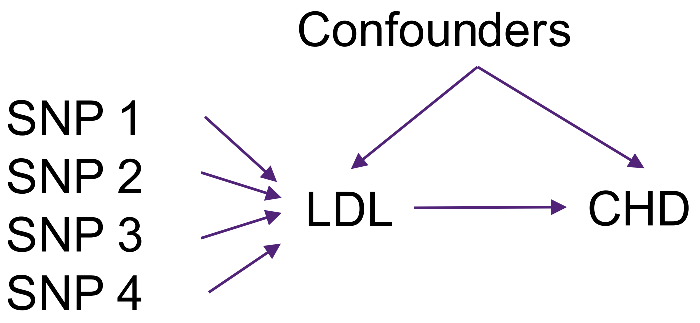


When there is <u>no horizontal pleiotropy</u>, slope = Causal effect ($\hat{\beta}_{XY}$)

Fixed effects IVW-MR and weighted linear regression



- IVW is equivalent to a weighted regression of SNP-outcome effects on SNP-exposure effects passing through the origin
- The weights are the inverse of the variance of the individual causal effect estimates, i.e., $\frac{1}{var(\widehat{\beta}_{XY_k})}$
- The slope is the estimate of the causal effect



Confounders

SNP 1
SNP 2
SNP 3
SNP 4

LDL

CHD

Assumptions for two-sample MR using multiple variants

- Using summarized data for two-sample MR analyses is convenient when sharing individual level data is impractical

- Two-sample MR can consistently estimate the true causal effect, if:
  - The k genetic variants are perfectly uncorrelated (SNPs not in LD) and do not interact
  - The two samples are homogenous (same underlying populations)
  - No sample overlap (this could be relaxed if all IVs are “valid”)
  - Constant causal effect at each level of the exposure

- Two-sample MR is still vulnerable to weak instrument bias

  - Bias towards the null effect, not the observational estimate

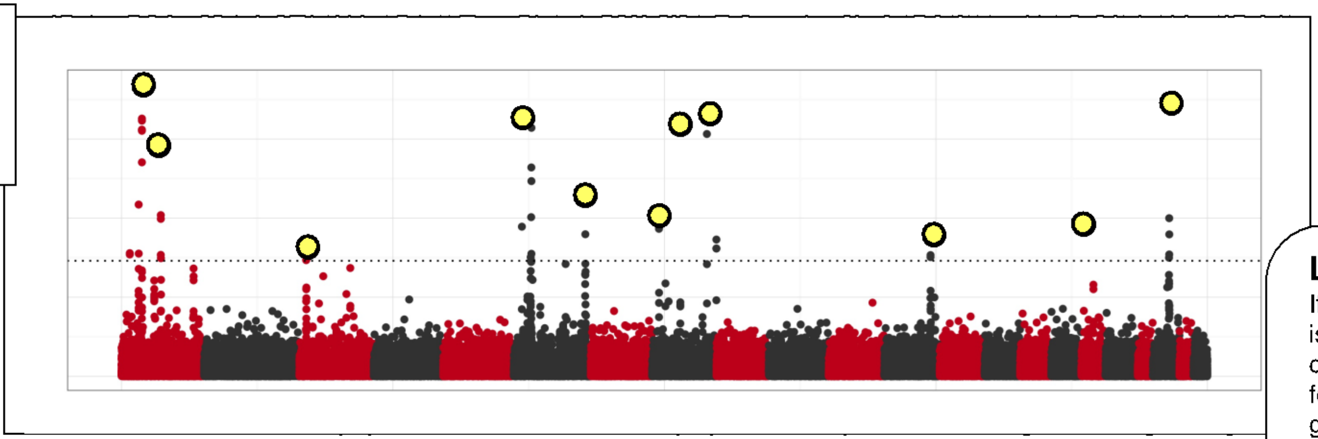
  - If approximate F-statistic ($\hat{\beta}^2_{Z_kX}/ \sigma^2_{Z_kX}$) is greater than 10, then the expected dilution of $\hat{\beta}_{XY_k}$ towards zero is less than 10%

The traditional rule of thumb

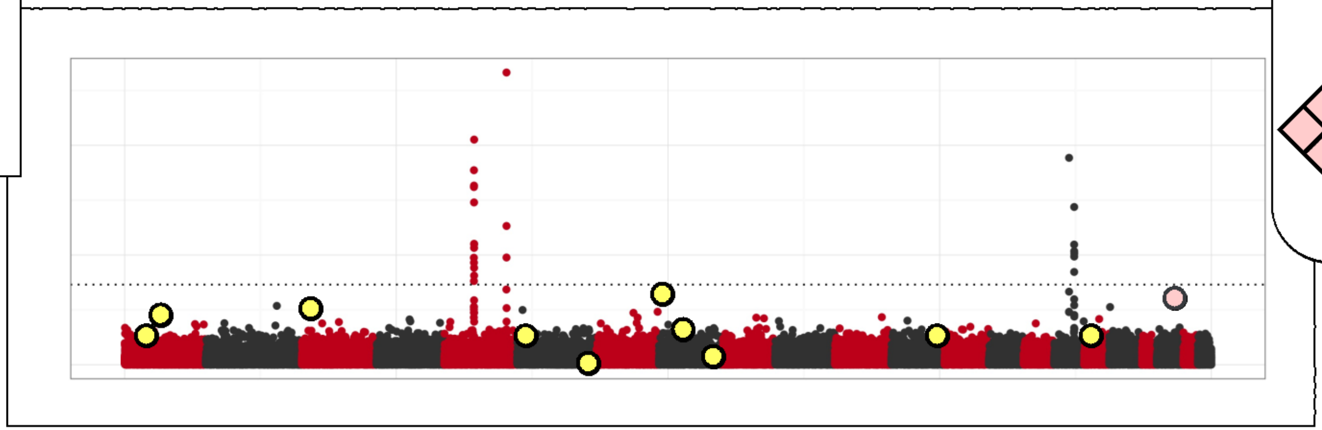
Performing two-sample MR with summary statistics

A convenient approach when sharing individual level data is impractical

Obtain instruments from exposure GWAS

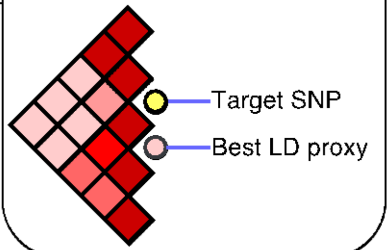


Extract SNP effects from outcome GWAS

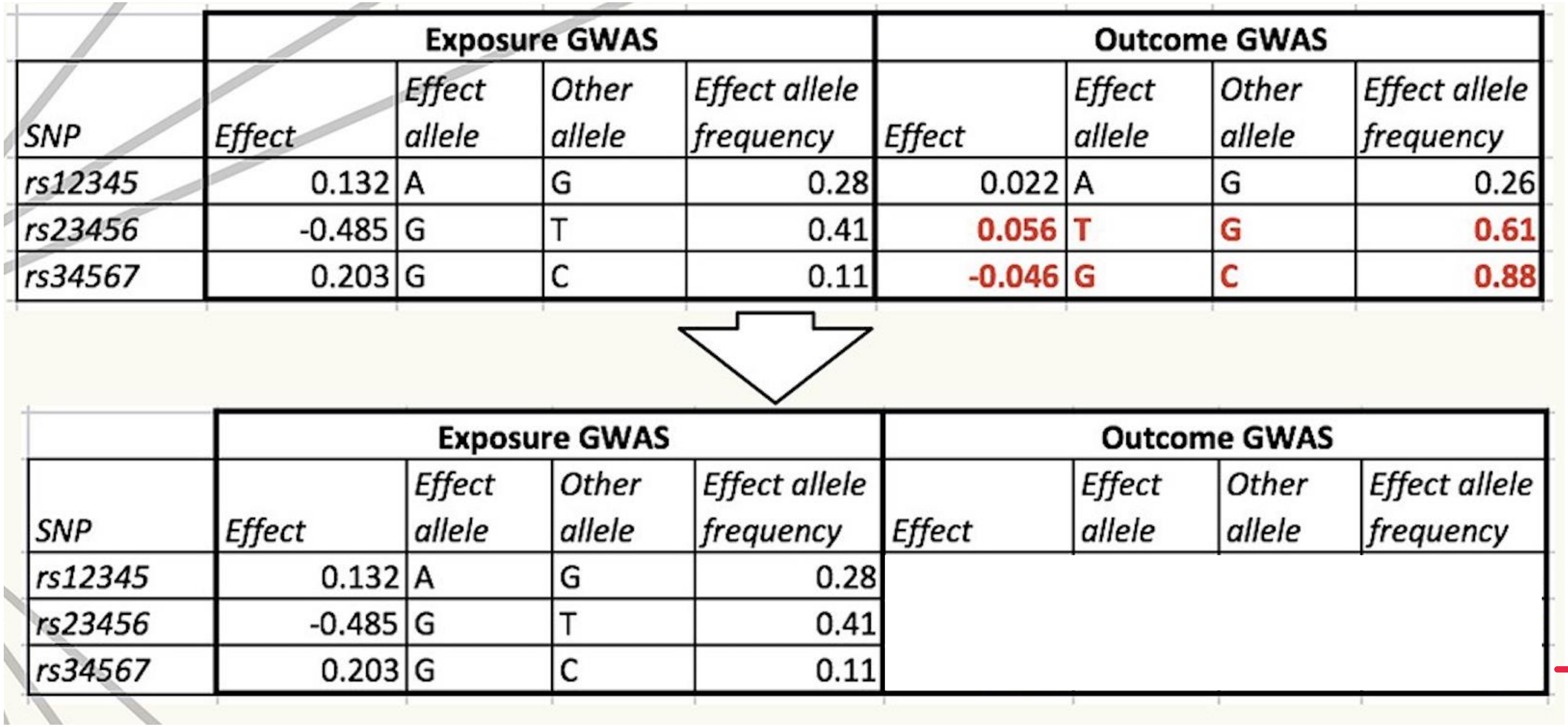


LD Proxies

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



Harmonise exposure and outcome effects



	Exposure GWAS				Outcome GWAS			
SNP	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*	*Effect*	*Effect allele*	*Other allele*	*Effect allele frequency*
rs12345	0.132	A	G	0.28	0.022	A	G	0.26
rs23456	-0.485	G	T	0.41	**0.056**	**T**	**G**	**0.61**
rs34567	0.203	G	C	0.11	**-0.046**	**G**	**C**	**0.88**

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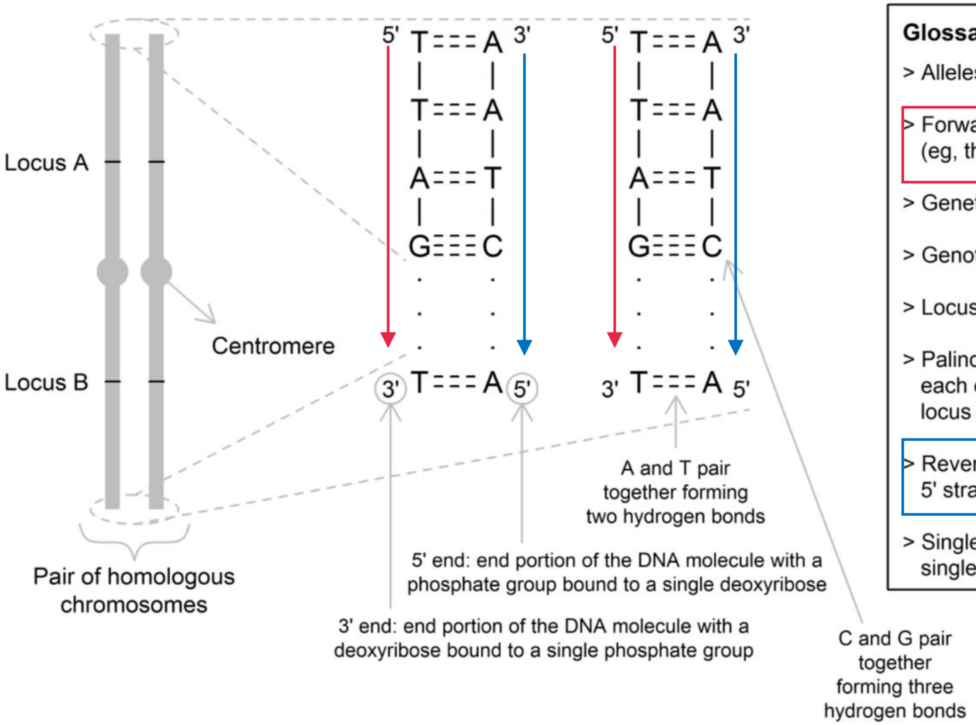
Palindromic

Palindromic SNP: The Issue of Strand



5' end: end portion of the DNA molecule with a phosphate group bound to a single deoxyribose

3' end: end portion of the DNA molecule with a deoxyribose bound to a single phosphate group

Glossary

> Alleles: variant forms that a locus may present.

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

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

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

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

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

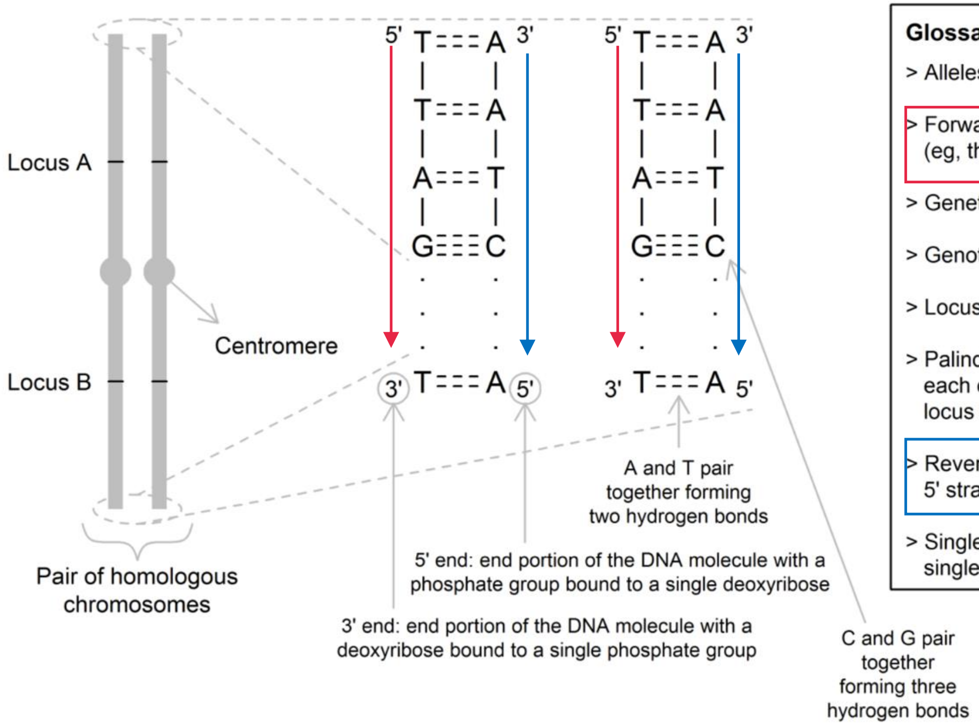
> Reverse or negative strand: the DNA strand that is read from the 3' to the 5' strand (eg, the 3' AATC...A 5' strand in the figure).

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

Exposure GWAS and outcome GWAS may be based on the genotypes of different strands.

Hartwig FP et al. *Int J Epidemiol.* (2016) 45(6):1717-1726

Palindromic SNP: The Issue of Strand



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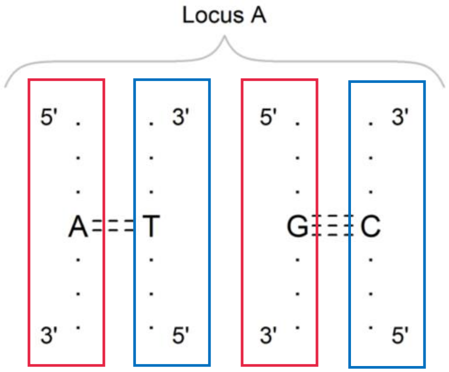
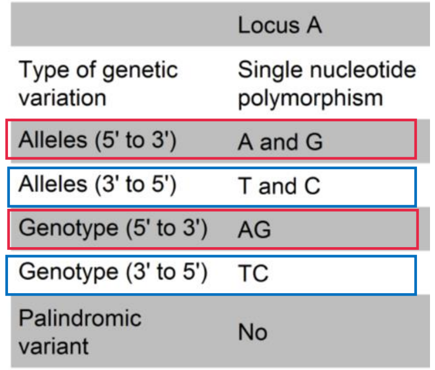
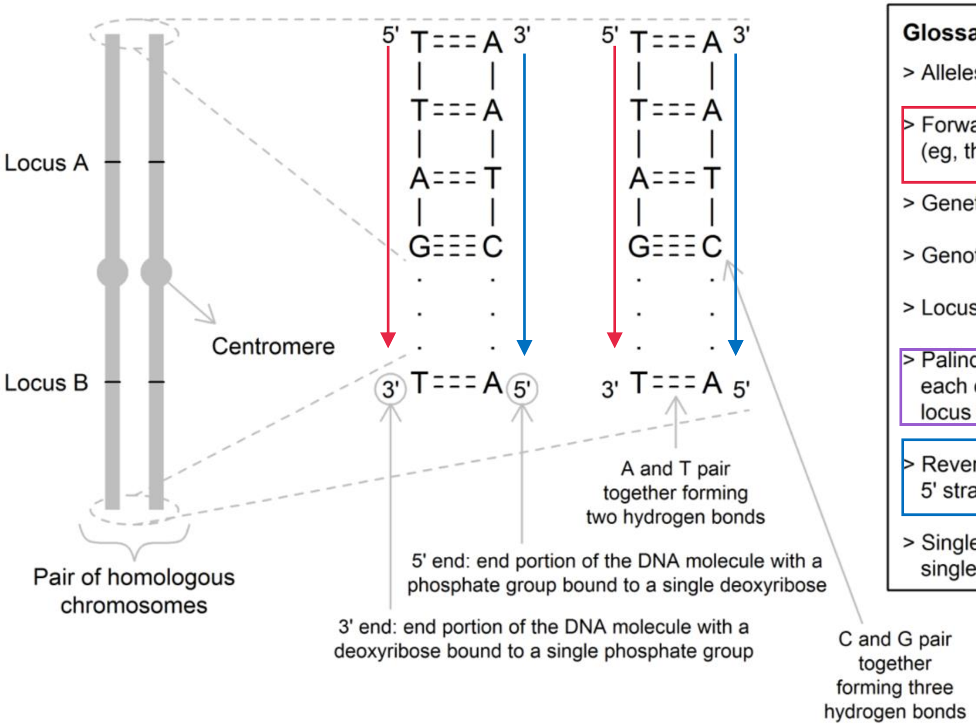




	Locus A
Type of genetic variation	Single nucleotide polymorphism
Alleles (5' to 3')	A and G
Alleles (3' to 5')	T and C
Genotype (5' to 3')	AG
Genotype (3' to 5')	TC
Palindromic variant	No

Hartwig FP et al. *Int J Epidemiol.* (2016) 45(6):1717-1726

Palindromic SNP: The Issue of Strand



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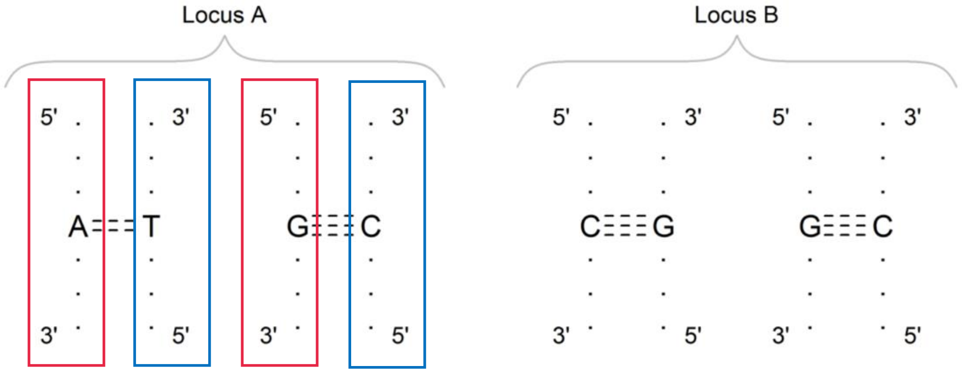
> Single nucleotide polymorphism (SNP): a type of genetic variant that involves single base pair changes.

Exposure GWAS and outcome GWAS may be based on the genotypes of different strands.

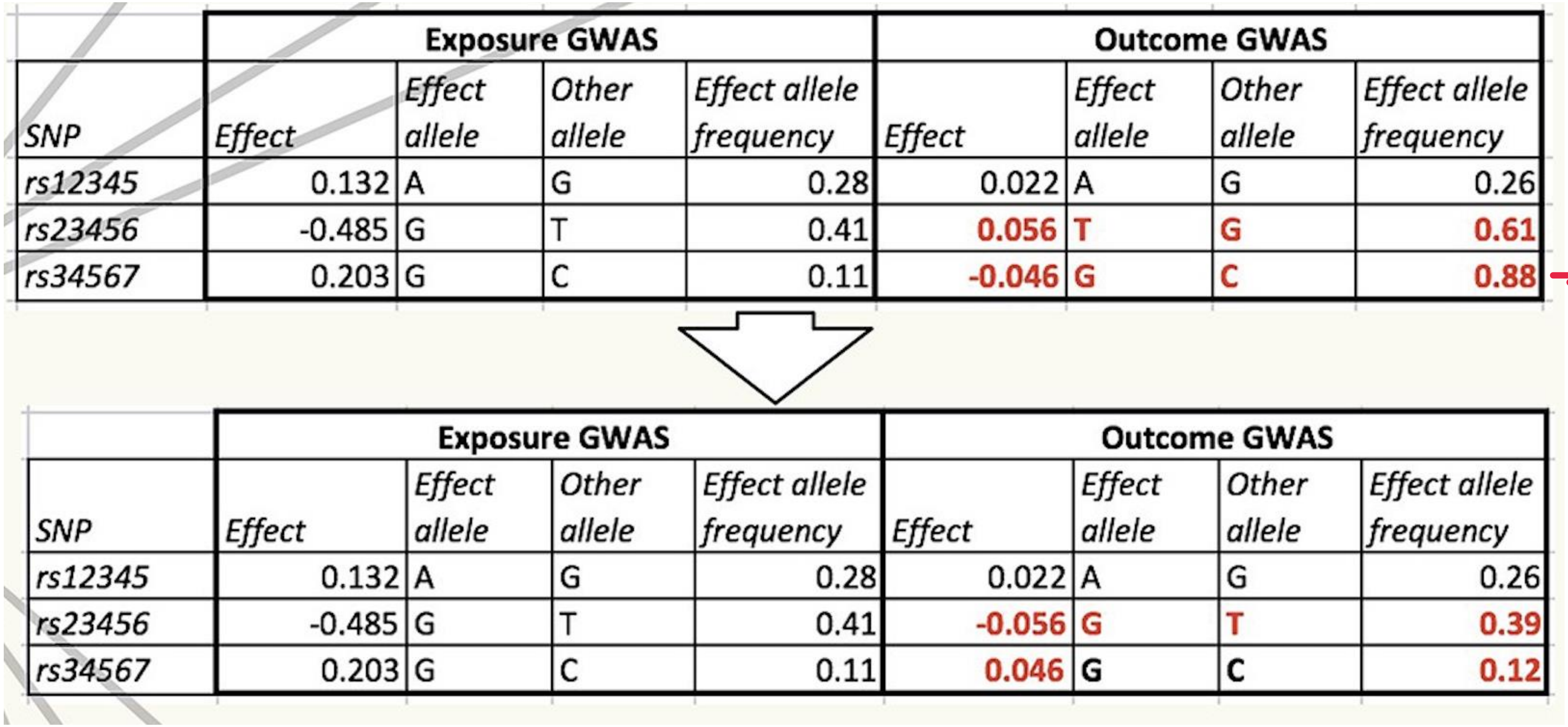


	Locus A	Locus B
Type of genetic variation	Single nucleotide polymorphism	Single nucleotide polymorphism
Alleles (5' to 3')	A and G	C and G
Alleles (3' to 5')	T and C	G and C
Genotype (5' to 3')	AG	CG
Genotype (3' to 5')	TC	GC
Palindromic variant	No	Yes

When there are palindromic SNPs, simply merging datasets based on effect alleles may result in the effect being the opposite.

Hartwig FP et al. *Int J Epidemiol.* (2016) 45(6):1717-1726

Harmonise exposure and outcome effects



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Palindromic

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rs23456	-0.485	G	T	0.41	**-0.056**	**G**	**T**	**0.39**
rs34567	0.203	G	C	0.11	**0.046**	**G**	**C**	**0.12**

Strand issue exercise

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

Break time

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WHAT IS THE PROBLEM WITH MR?

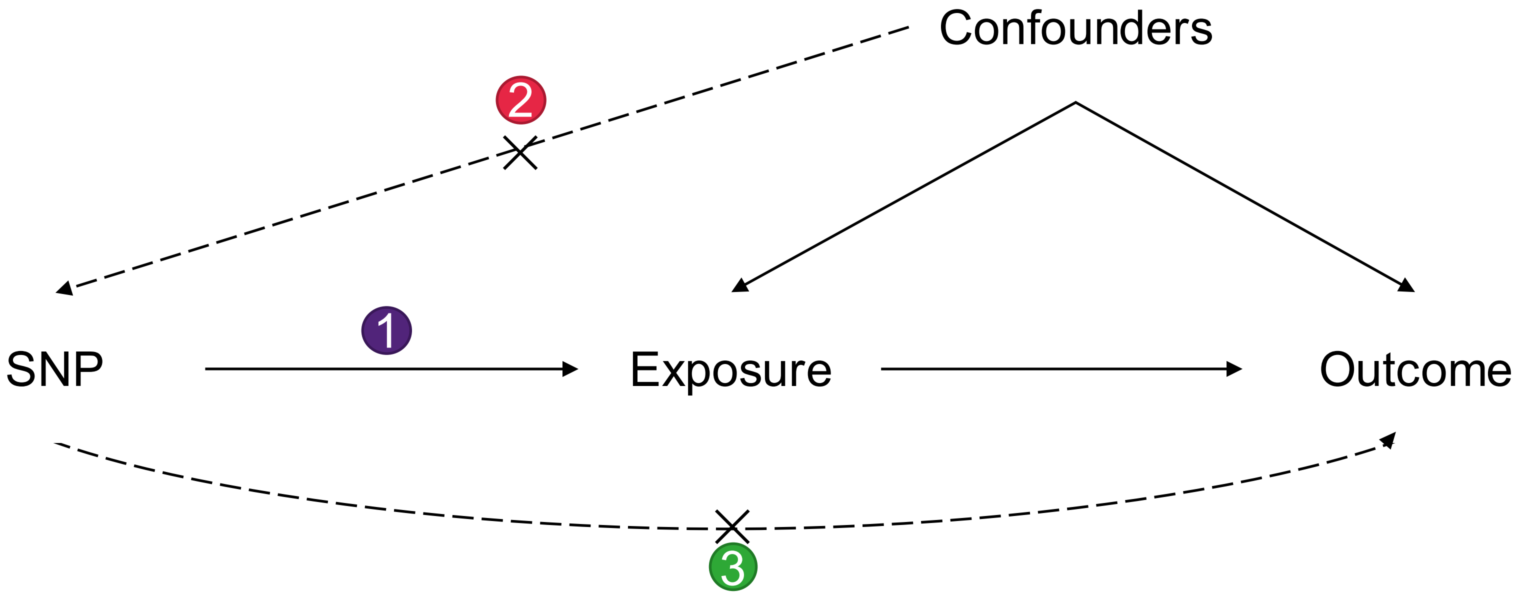
- MR uses genetic variants to test for causal relationships between phenotypic exposures and disease-related outcomes

- 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 **horizontal 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

Three core MR assumptions



(1) Relevance assumption: SNP is associated with the exposure

(2) Independence assumption: There are no confounding associations between the instrumental variables (IVs) and the outcome.

👉 (3) Exclusion restriction: SNP ONLY associated outcome through the exposure

Many MR methods for handling horizontal pleiotropy

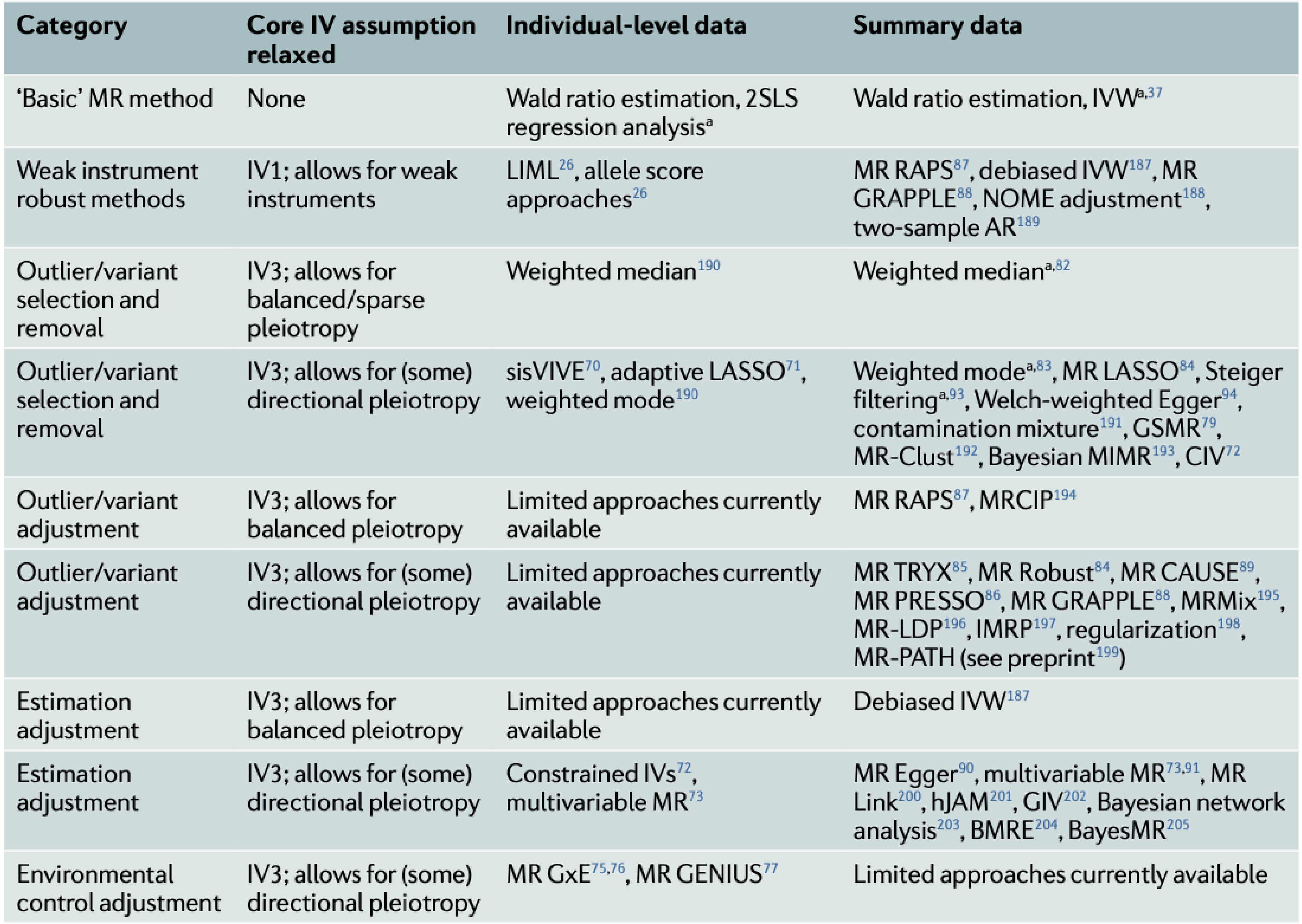


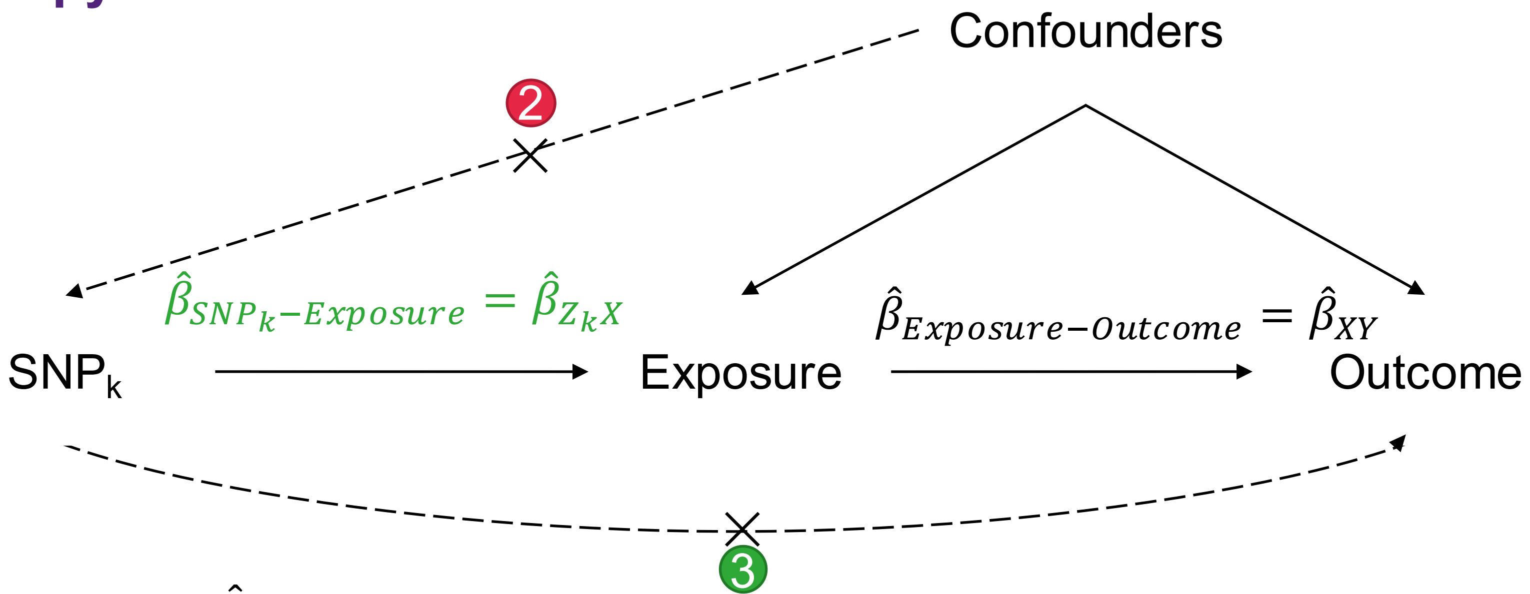
Table 1 | **List of MR estimation methods**

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

Sanderson et al. *Nat Rev Methods Primers* 2022

Two-sample MR

No direct pleiotropy



Confounders

②

$\hat{\beta}_{SNP_k-Exposure} = \hat{\beta}_{Z_kX}$

$\hat{\beta}_{Exposure-Outcome} = \hat{\beta}_{XY}$

SNP$_k$ Exposure Outcome

③

$\hat{\beta}_{SNP-Outcome} = \hat{\beta}_{SNP-Exposure} \times \hat{\beta}_{Exposure-Outcome}$

Causal effect by Wald estimator:

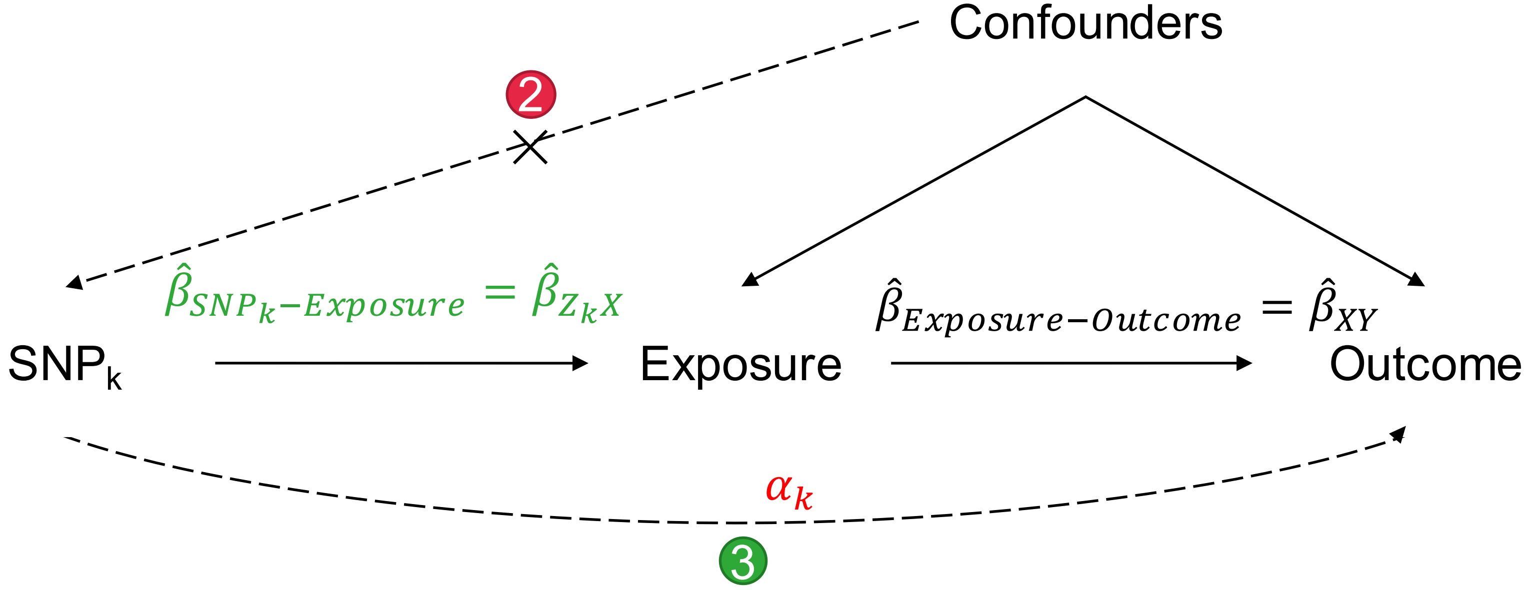
$$\hat{\beta}_{XY_k} = \frac{\hat{\beta}_{SNP_k-Outcome}}{\hat{\beta}_{SNP_k-Exposure}}$$

Inverse variance weighted (IVW) average causal effect:

$$\hat{\beta}_{IVW} = \frac{\sum_{k=1}^{K} \hat{\beta}_{XY_k} w_k}{\sum_{k=1}^{K} w_k}$$

Two-sample MR

With direct pleiotropy (α_k)



$$\hat{\beta}_{SNP-Outcome} = \hat{\beta}_{SNP-Exposure} \times \hat{\beta}_{Exposure-Outcome} + \alpha_k$$

Causal effect by Wald estimator:

$$\frac{\widehat{\beta}_{SNP_k-Outcome}}{\widehat{\beta}_{SNP_k-Exposure}} = \hat{\beta}_{Exposure-Outcome} + \frac{\alpha_k}{\widehat{\beta}_{SNP_k-Exposure}}$$

Inverse variance weighted (IVW) average causal effect:

$$\frac{\sum_{k=1}^{K} \widehat{\beta}_{XY_k} w_k}{\sum_{k=1}^{K} w_k} = \hat{\beta}_{Exposure-Outcome} + \text{Bias}\,(\alpha,\ \hat{\beta}_{SNP_k-Exposure})$$

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 (heterogeneity test):

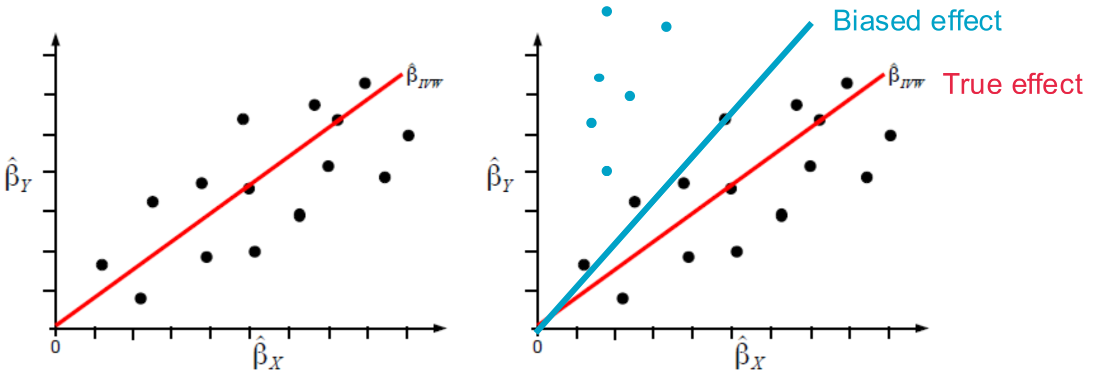
$$Q = \sum_{k=1}^{K} w_k \left(\hat{\beta}_{XY_k} - \hat{\beta}_{IVW}\right)^2$$

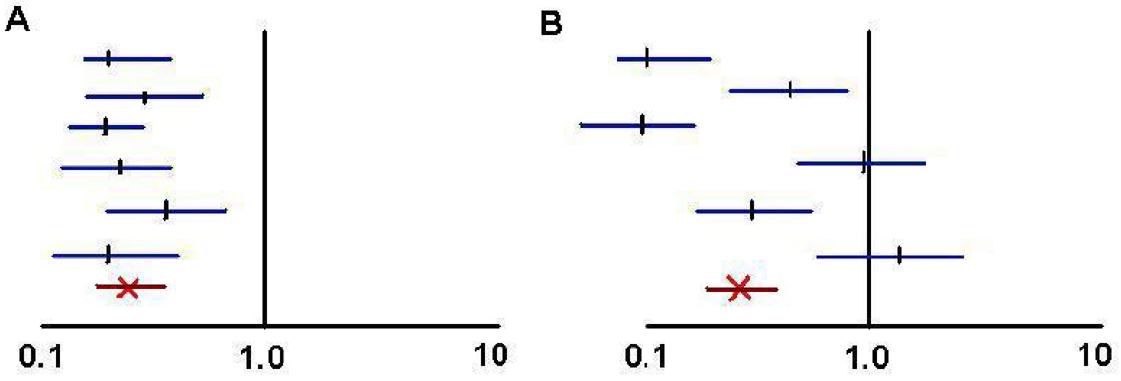
Where w_k is the weight (i.e. inversed variance) of the causal estimate at SNP *k*.

If MR model is correct, *Q* follows a χ^2 distribution with expected value *K*-1.

If *Q* is larger than *K*-1, then the estimates exhibit over-dispersion.

- ● SNPs are valid instruments
- ● SNPs associated with outcome via an independent pathway.





N=6 instruments

(A): No heterogeneity; all variants estimating the same quantity: $Q \approx 5$

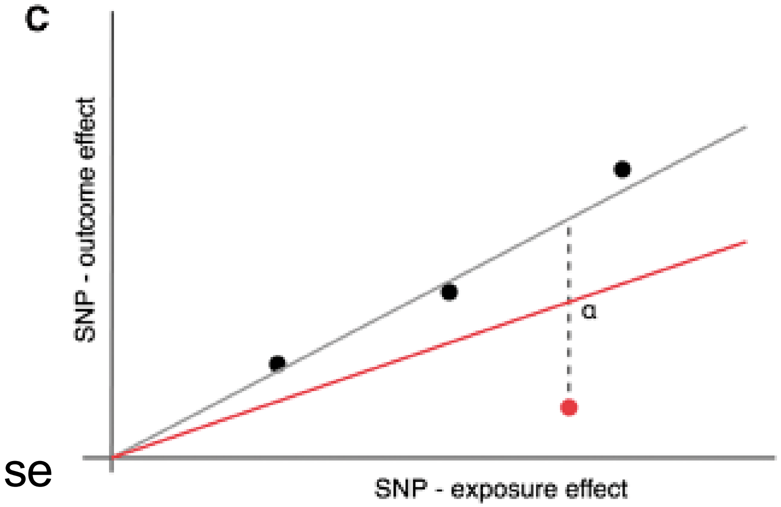
(B): Heterogeneity; variants estimating different quantities: $Q >> 5$

Bowden et al. Am J Epidemiol. (2018) 187(12):2681-2685

Accounting for heterogeneity

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?

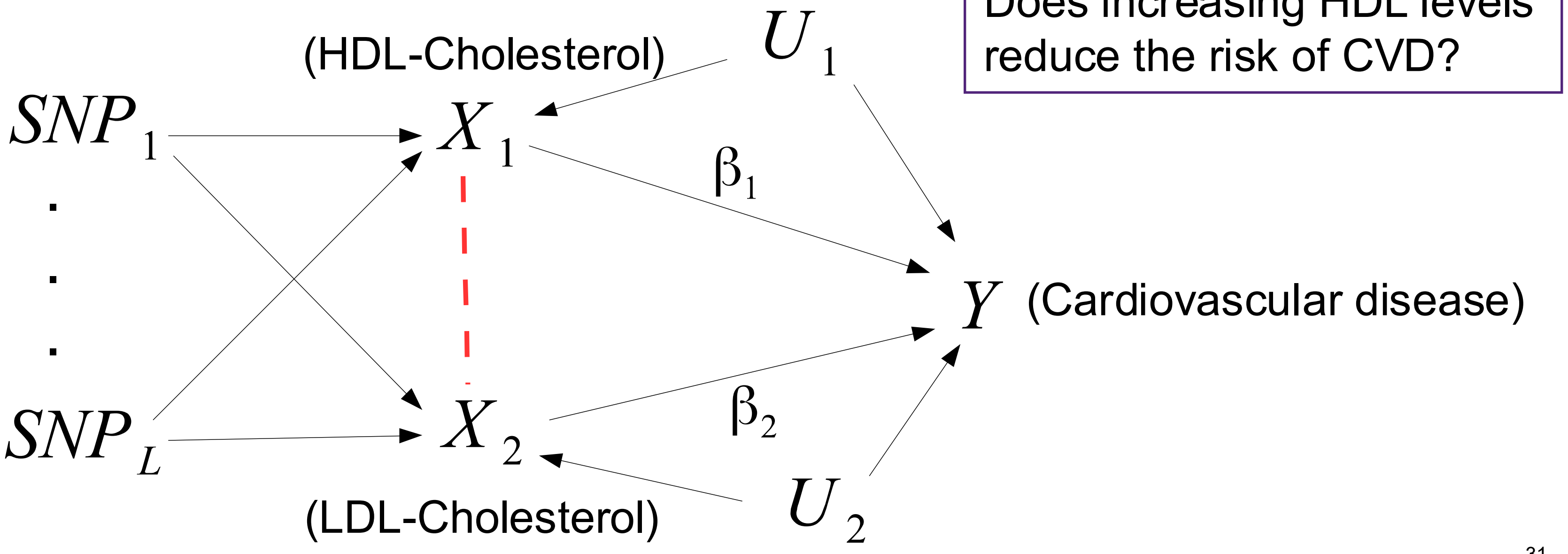
- E.g. cis-variants for gene expression, DNA methylation, and protein levels. CRP levels are best instrumented by variants within the *CRP* gene region. Most other variants that come up in CRP GWAS are upstream effects related to inflammation.

Accounting for heterogeneity

Option 2: Multivariable MR

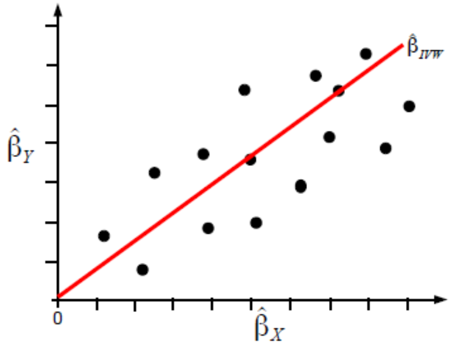
- We are testing for whether X_1 has an influence on Y
- We know that some instruments for X_1 also have influences on X_2
- This opens up the possibility of horizontal pleiotropy biasing our estimate
- What is the X_1-Y association adjusting for X_2?

Does increasing HDL levels reduce the risk of CVD?



Accounting for heterogeneity

Option 3: Fit a model that is robust to certain types of horizontal pleiotropy



- IVW fixed effects estimator assumes all SNPs are valid instruments and averages across them all.
  - Clear trend in estimates increasing with $\hat{\beta}_{Z_k X}$ from origin
  - Cochran's $Q \approx K - 1$ (no heterogeneity)

- If there is an indication that these don't hold in the data, invalid "pleiotropic" variants could be the cause.

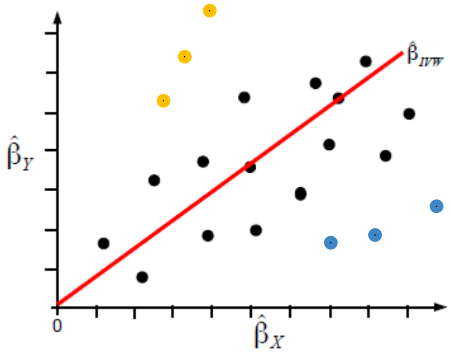
- Can the IVW method still estimate the causal effect without bias even when all variants have pleiotropic effects? Yes, if pleiotropy is balanced.

Accounting for heterogeneity

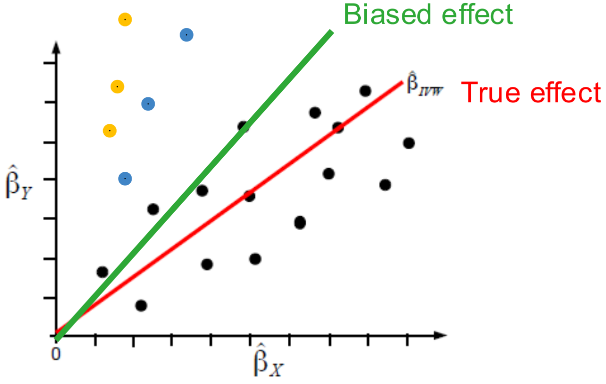
Option 3: Fit a model that is robust to certain types of horizontal pleiotropy

IVW random effects estimator allows all SNPs to be invalid due to pleiotropy as long as the pleiotropy is balanced.

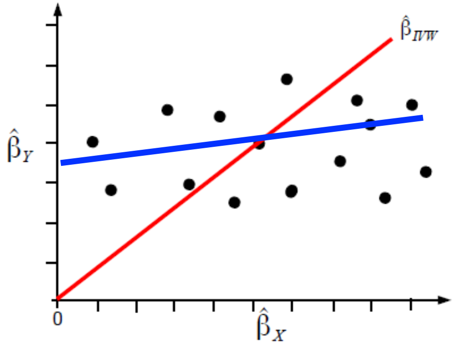
- The standard error of the causal estimate increases with the degree of heterogeneity.



Balanced



Imbalanced
(zero-intercept)



Imbalanced

We could therefore regress the SNP-outcome associations on the SNP-exposure associations, but allow for a **non-zero intercept** in the regression This is the principal behind **MR-Egger regression**.

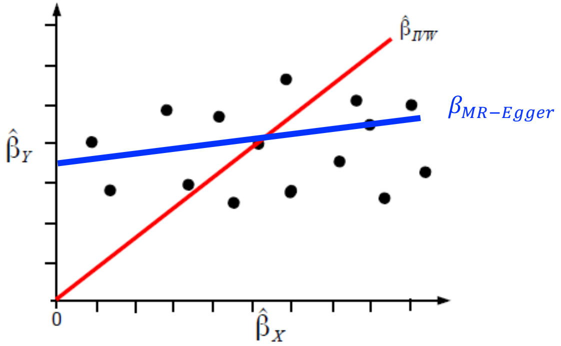
● SNPs are valid instruments
● SNP associated with outcome via confounder.
● SNP associated with outcome via an independent pathway.

Trend away from origin + heterogeneity
- Zero-intercept condition unreasonable
- IVW does not appear to be a good fit

Pleiotropy potentially causes heterogeneity and bias

MR-Egger regression: Central concept

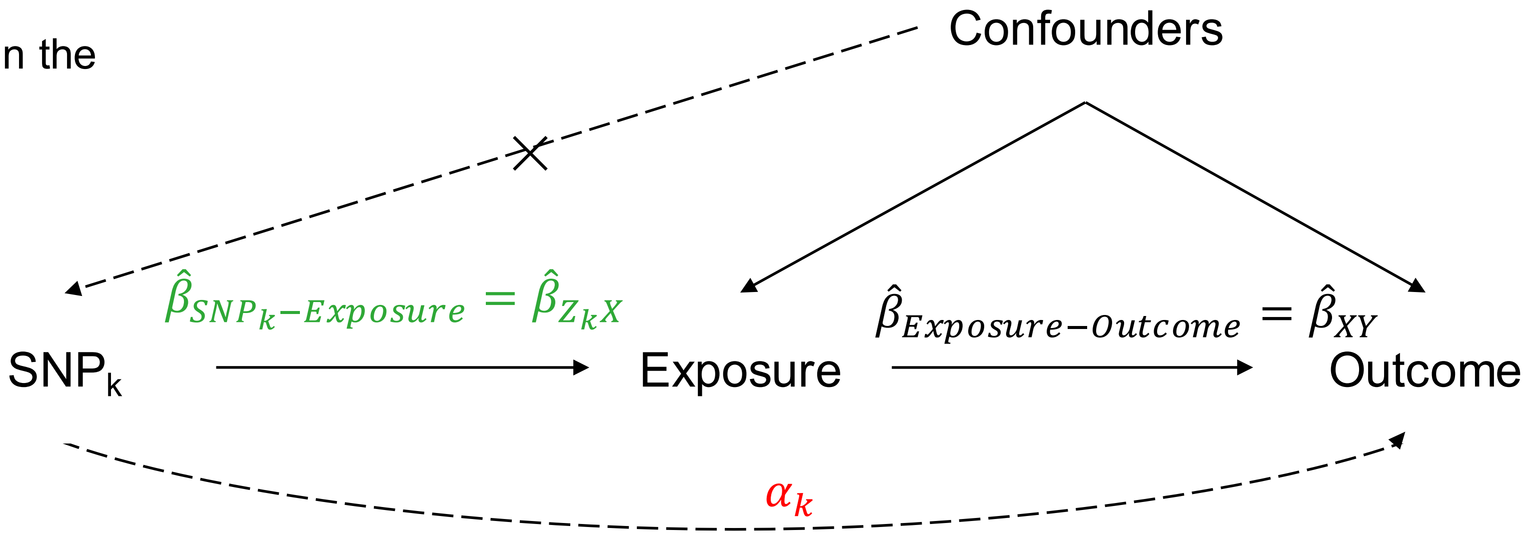
MR-Egger allows for a **non-zero intercept** in the regression.



When multiple SNPs are used as instruments, MR-Egger can:

- Identify the presence of “directional” pleiotropy (biasing the causal estimate in IVW)
- Provide a less biased causal estimate (in the presence of pleiotropy)

MR-Egger lacks power.


Confounders

$\hat{\beta}_{SNP_k-Exposure} = \hat{\beta}_{Z_kX}$

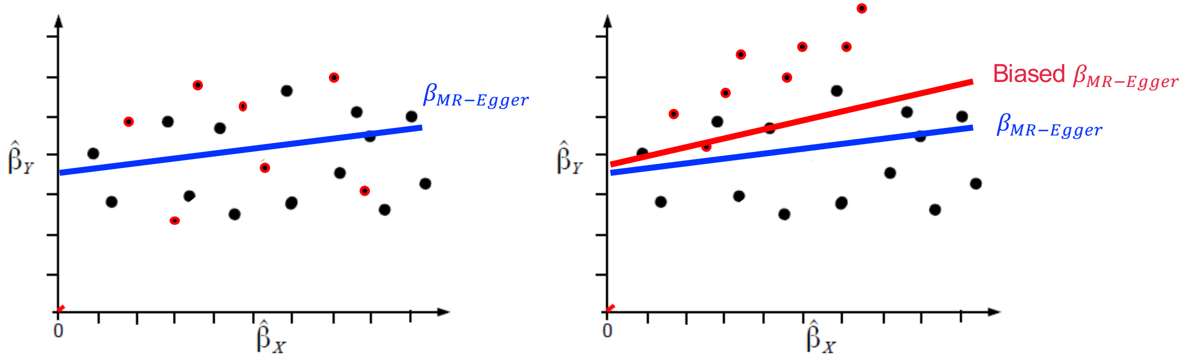
$\hat{\beta}_{Exposure-Outcome} = \hat{\beta}_{XY}$

SNP$_k$ Exposure Outcome

α_k

MR-Egger regression

MR-Egger regression replies on the InSIDE (INstrument Strength Independent of Direct Effect) assumption, which states that the pleiotropic effects of SNPs must be independent of their strength as instruments.



InSIDE

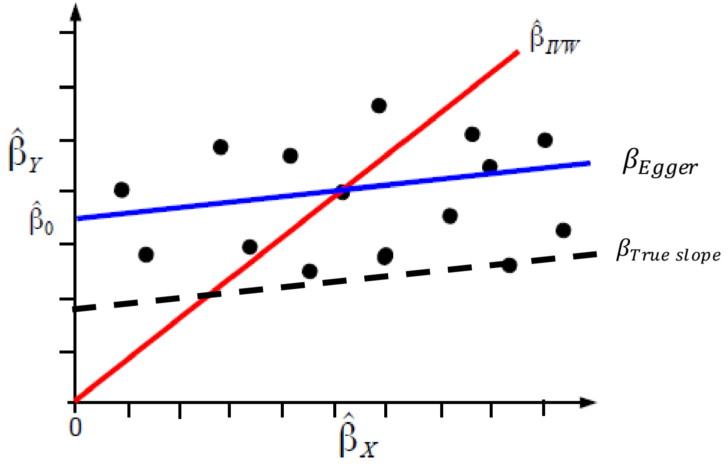
Violation of InSIDE

- ● SNP not associated with outcome via an independent pathway
- ● SNP associated with outcome via an independent pathway

IVW model: $\hat{\beta}_{Yk} = \underbrace{\beta_{IVW}}_{\text{Slope}} \hat{\beta}_{Xk} + + \varepsilon_{Yk}$

MR-Egger model: $\hat{\beta}_{Yk} = \beta_0 + \underbrace{\beta_{Egger}}_{\text{Slope}} \hat{\beta}_{Xk} + \varepsilon_{Yk}$

- β_0 is the intercept term. β_0 can be interpreted as the average pleiotropic effect across all genetic variants. A non-zero β_{0E} indicates directional pleiotropy.
- β_{Egger} is the causal estimate adjusted for directional pleiotropy



Bowden et al. Int J Epidemiol. (2015) 44(2):512-25

Median based methods (Median Estimator)

Order causal estimates (Wald ratio) and take the median.

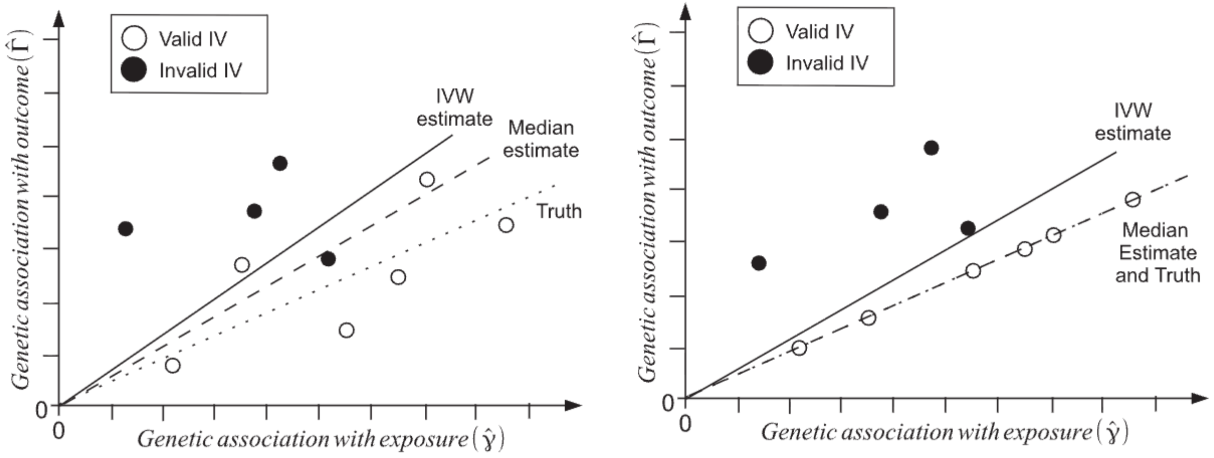


Figure 2. Fictional example of a Mendelian randomization analysis with 10 genetic variants—six valid instrumental variables (hollow circles) and four invalid instrumental variables (solid circles) for finite sample size (left) and infinite sample size (right) showing IVW (solid line) and simple median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

Assumption: >50% of the instrumental variables are valid.

No restrictions need to be placed on the invalid IVs:

- InSIDE assumption not required
- Violations of #2 and #3 MR assumptions are allowed

Bowden et al. Genet Epidemiol. (2016) 40(4):304-314

Median based methods

Simple median estimator

- Odd number of IVs: middle ratio estimate
- Even number of IVs: median is the average of the two middle estimates $\left(\frac{1}{2}\left(\hat{\beta}_k + \hat{\beta}_{k+1}\right)\right)$
    - Inefficient when the precision of individual variants varies considerably

	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$	$\hat{\beta}_4$	$\hat{\beta}_5$	$\hat{\beta}_6$	$\hat{\beta}_7$	$\hat{\beta}_8$	$\hat{\beta}_9$	$\hat{\beta}_{10}$
Simple median										
Weight $(1/v_k)$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$	$\frac{1}{10}$
Percentile (p_k)	5	15	25	35	45	55	65	75	85	95

50 %

$$\hat{\beta}_{Simple\ median} = \frac{\hat{\beta}_5 + \hat{\beta}_6}{2}$$

Bowden et al. Genet Epidemiol. (2016) 40(4):304-314

Median based methods

Weighted median estimator

- Weighted median estimator takes into account the differing precisions
- Weighted median: $\hat{\beta}_{WM} = \hat{\beta}_3 + \left(\hat{\beta}_4 - \hat{\beta}_3\right) \times \frac{50-27.78}{52.78-27.78}$
- Suggested weights: inversed variance of the ratio estimate: $w'_k = \frac{1}{\boldsymbol{var}(\hat{\beta}_{XY_k})}$

	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$	$\hat{\beta}_4$	$\hat{\beta}_5$	$\hat{\beta}_6$	$\hat{\beta}_7$	$\hat{\beta}_8$	$\hat{\beta}_9$	$\hat{\beta}_{10}$
Simple median Weight $(1/v_k)$ Percentile (p_k)	$\frac{1}{10}$ 5	$\frac{1}{10}$ 15	$\frac{1}{10}$ 25	$\frac{1}{10}$ 35	$\frac{1}{10}$ 45 50 %	$\frac{1}{10}$ 55	$\frac{1}{10}$ 65	$\frac{1}{10}$ 75	$\frac{1}{10}$ 85	$\frac{1}{10}$ 95
Weighting 1 Weight $(1/v_k)$ Percentile	$\frac{1}{30}$ 1.67	$\frac{2}{30}$ 6.67	$\frac{3}{30}$ 15.00	$\frac{4}{30}$ 26.67	$\frac{5}{30}$ 41.67 50 %	$\frac{5}{30}$ 58.33	$\frac{4}{30}$ 73.33	$\frac{3}{30}$ 85.00	$\frac{2}{30}$ 93.33	$\frac{1}{30}$ 98.33
Weighting 2 Weight $(1/v_k)$ Percentile (p_k)	$\frac{2}{36}$ 2.78	$\frac{3}{36}$ 9.72	$\frac{10}{36}$ 27.78 50 %	$\frac{8}{36}$ 52.78	$\frac{5}{36}$ 70.83	$\frac{3}{36}$ 81.94	$\frac{2}{36}$ 88.89	$\frac{1}{36}$ 93.06	$\frac{1}{36}$ 95.83	$\frac{1}{36}$ 98.61

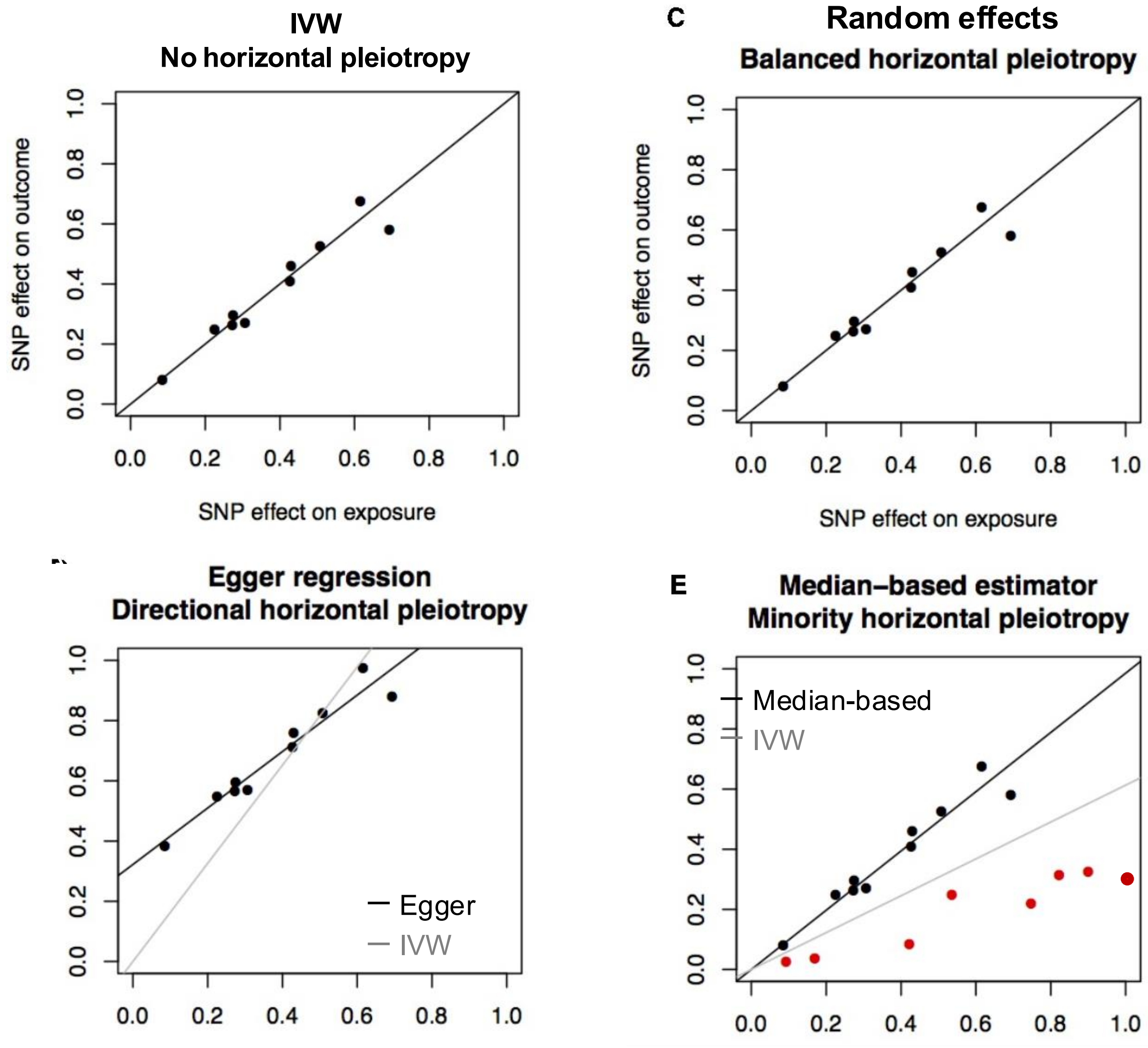
$$\hat{\beta}_{Simple\ median} = \frac{\hat{\beta}_5+\hat{\beta}_6}{2}$$

$$\hat{\beta}_{WM} = \hat{\beta}_5 + \left(\hat{\beta}_6 - \hat{\beta}_5\right) \times \frac{50 - 41.67}{58.33 - 41.67}$$

$$\hat{\beta}_{WM} = \hat{\beta}_3 + \left(\hat{\beta}_4 - \hat{\beta}_3\right) \times \frac{50 - 27.78}{52.78 - 27.78}$$

Bowden et al. Genet Epidemiol. (2016) 40(4):304-314

Summary of robust estimators



IVW
No horizontal pleiotropy

C Random effects
Balanced horizontal pleiotropy

Egger regression
Directional horizontal pleiotropy

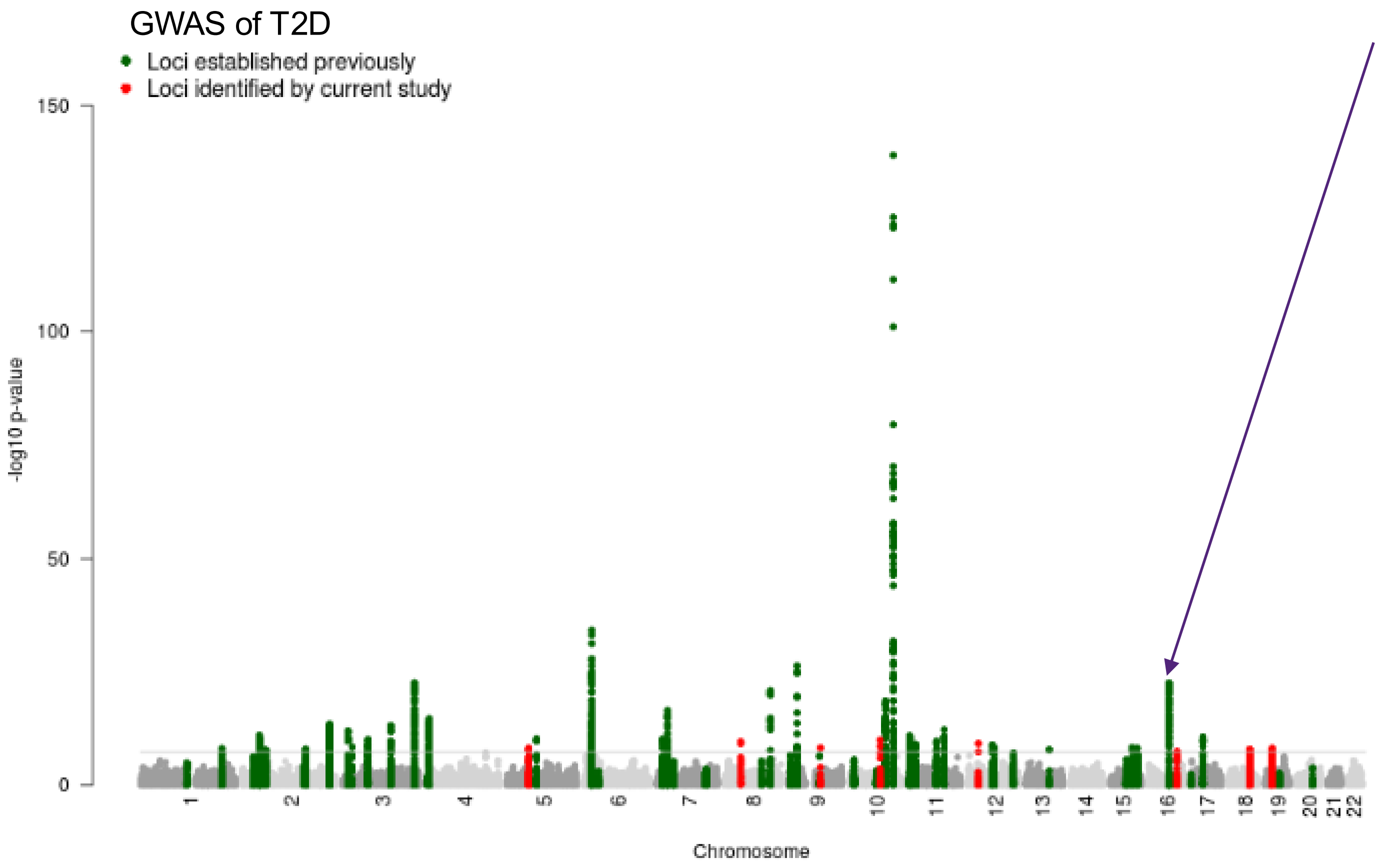
E Median–based estimator
Minority horizontal pleiotropy

SNPs associated with outcome via an independent pathway.

Hemani et al. eLife (2018)

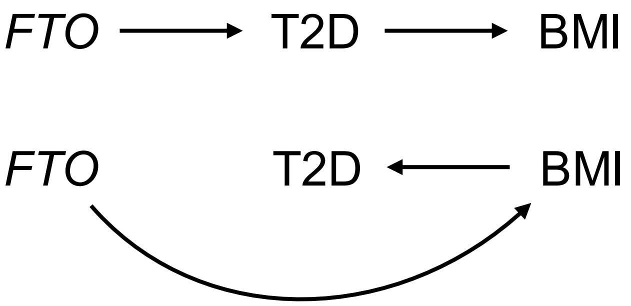
Reverse causal instruments

Problem: MR of type 2 diabetes on BMI



GWAS of T2D reveals *FTO* variant

- Famously associated with BMI
- A reverse causal instrument?

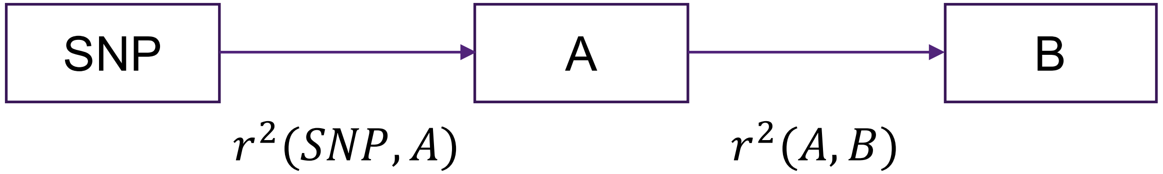


FTO may be associated with T2D through its effect on BMI
>> Not a good instrument for T2D

Can we avoid including reverse-causal SNPs as instruments?

Steiger filtering test

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

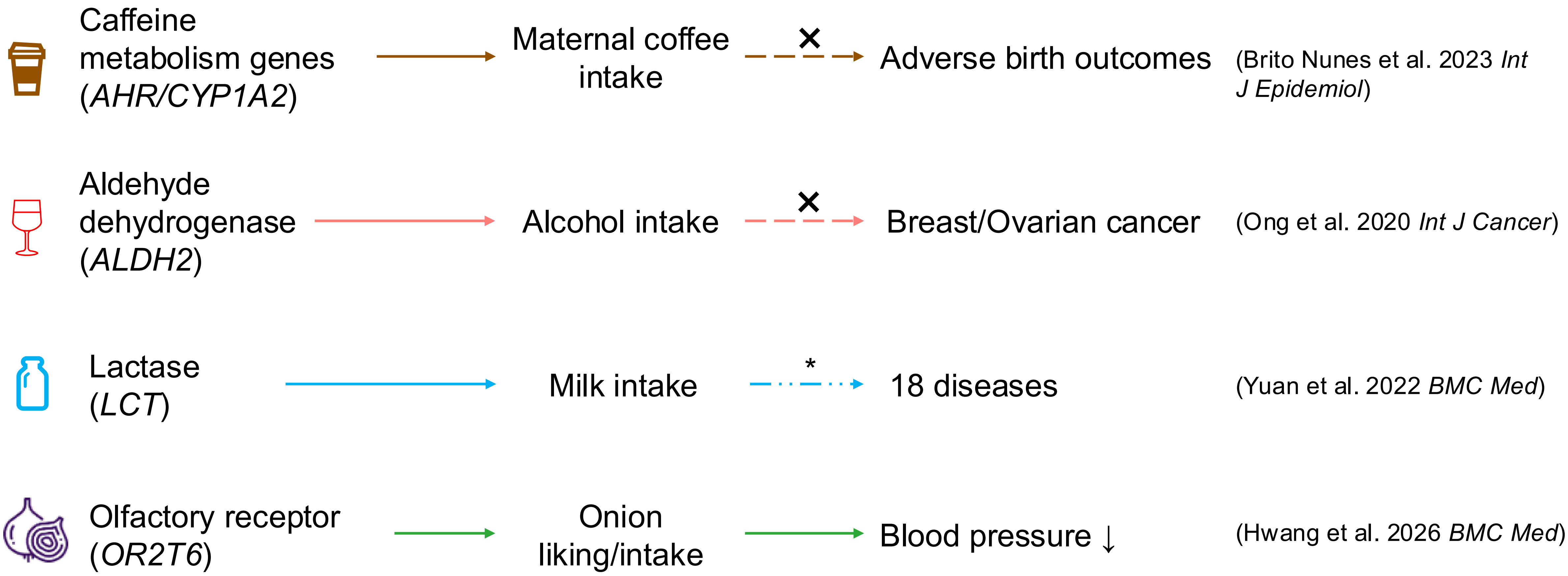


Expect that

$$r^2(SNP, B) = r^2(SNP, A) \times \underbrace{r^2(A, B)}_{\text{This term is <1}}$$

- Steiger test used to evaluate if r^2(SNP,A) > r^2(SNP,B)
- If this is not satisfied, infer that this instrument is not influencing the exposure primarily.

Ideal instruments are genetic variants with a known biological function related to the exposure



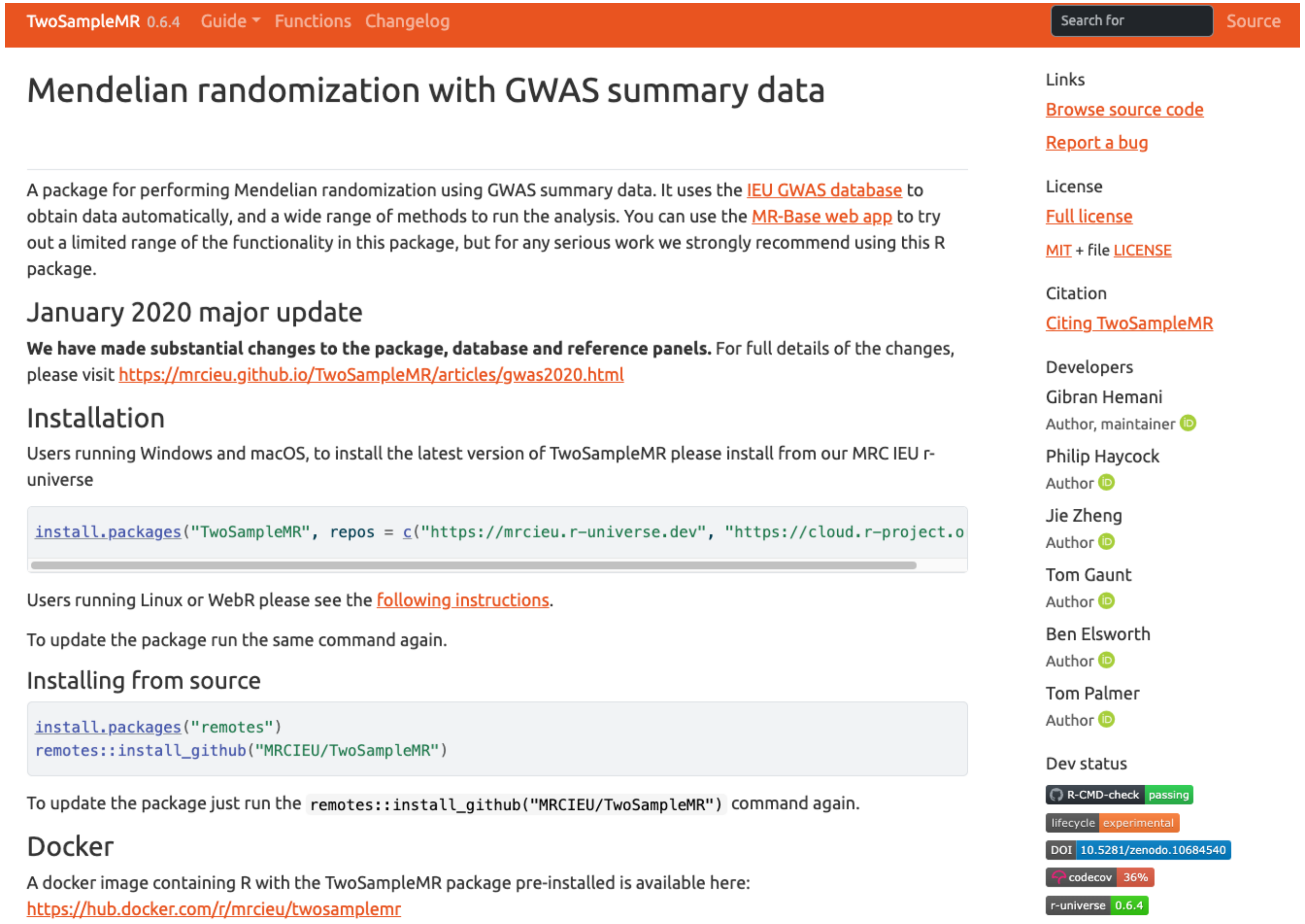
Caffeine metabolism genes (*AHR/CYP1A2*) → Maternal coffee intake ⇢ ✕ ⇢ Adverse birth outcomes (Brito Nunes et al. 2023 *Int J Epidemiol*)

Aldehyde dehydrogenase (*ALDH2*) → Alcohol intake ⇢ ✕ ⇢ Breast/Ovarian cancer (Ong et al. 2020 *Int J Cancer*)

Lactase (*LCT*) → Milk intake ⇢ * ⇢ 18 diseases (Yuan et al. 2022 *BMC Med*)

Olfactory receptor (*OR2T6*) → Onion liking/intake → Blood pressure ↓ (Hwang et al. 2026 *BMC Med*)

TwoSampleMR R Package



TwoSampleMR 0.6.4 Guide Functions Changelog Search for Source

Mendelian randomization with GWAS summary data

A package for performing Mendelian randomization using GWAS summary data. It uses the IEU GWAS database to obtain data automatically, and a wide range of methods to run the analysis. You can use the MR-Base web app to try out a limited range of the functionality in this package, but for any serious work we strongly recommend using this R package.

January 2020 major update

We have made substantial changes to the package, database and reference panels. For full details of the changes, please visit https://mrcieu.github.io/TwoSampleMR/articles/gwas2020.html

Installation

Users running Windows and macOS, to install the latest version of TwoSampleMR please install from our MRC IEU r-universe

```
install.packages("TwoSampleMR", repos = c("https://mrcieu.r-universe.dev", "https://cloud.r-project.o
```

Users running Linux or WebR please see the following instructions.

To update the package run the same command again.

Installing from source

```
install.packages("remotes")
remotes::install_github("MRCIEU/TwoSampleMR")
```

To update the package just run the `remotes::install_github("MRCIEU/TwoSampleMR")` command again.

Docker

A docker image containing R with the TwoSampleMR package pre-installed is available here: https://hub.docker.com/r/mrcieu/twosamplemr

Links

Browse source code

Report a bug

License

Full license

MIT + file LICENSE

Citation

Citing TwoSampleMR

Developers

Gibran Hemani
Author, maintainer

Philip Haycock
Author

Jie Zheng
Author

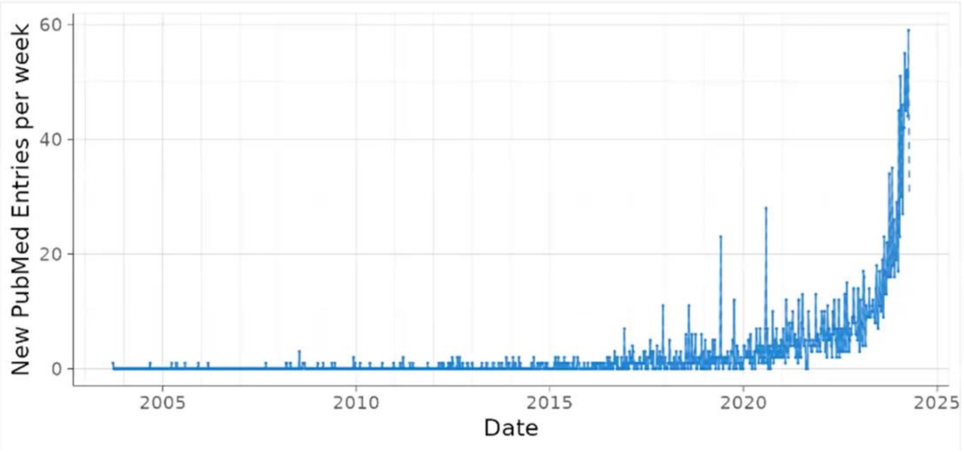
Tom Gaunt
Author

Ben Elsworth
Author

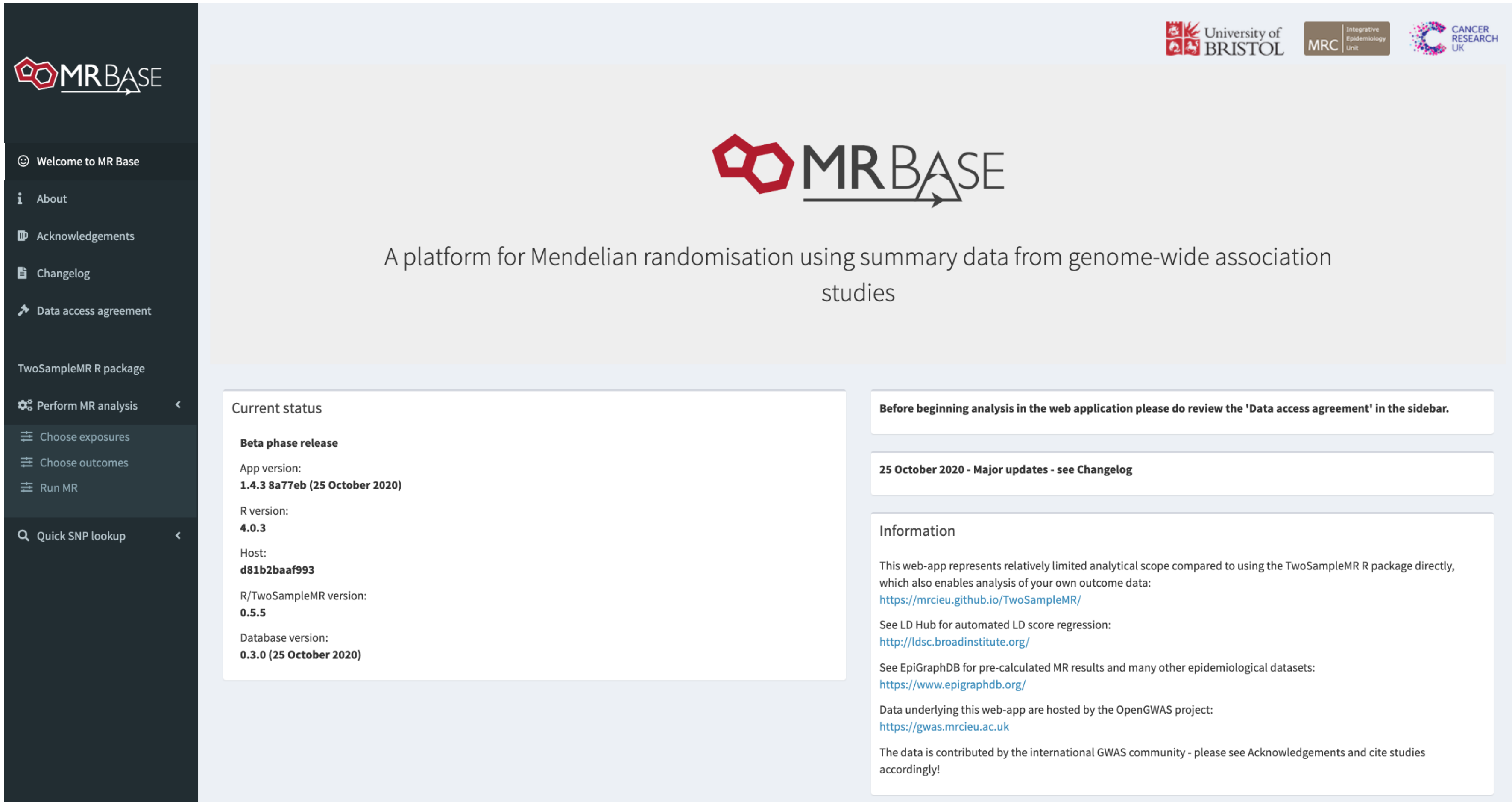
Tom Palmer
Author

Dev status

R-CMD-check passing
lifecycle experimental
DOI 10.5281/zenodo.10684540
codecov 36%
r-universe 0.6.4



MR Base (http://www.mrbase.org/)



A platform for Mendelian randomisation using summary data from genome-wide association studies

Current status

Beta phase release

App version:
1.4.3 8a77eb (25 October 2020)

R version:
4.0.3

Host:
d81b2baaf993

R/TwoSampleMR version:
0.5.5

Database version:
0.3.0 (25 October 2020)

Before beginning analysis in the web application please do review the 'Data access agreement' in the sidebar.

25 October 2020 - Major updates - see Changelog

Information

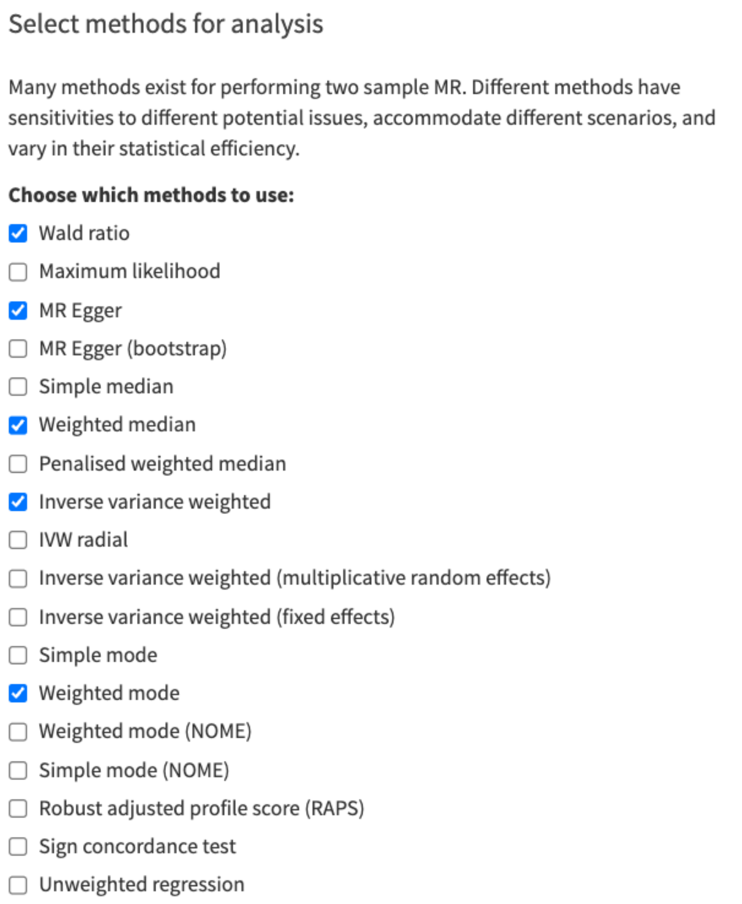
This web-app represents relatively limited analytical scope compared to using the TwoSampleMR R package directly, which also enables analysis of your own outcome data:
https://mrcieu.github.io/TwoSampleMR/

See LD Hub for automated LD score regression:
http://ldsc.broadinstitute.org/

See EpiGraphDB for pre-calculated MR results and many other epidemiological datasets:
https://www.epigraphdb.org/

Data underlying this web-app are hosted by the OpenGWAS project:
https://gwas.mrcieu.ac.uk

The data is contributed by the international GWAS community - please see Acknowledgements and cite studies accordingly!



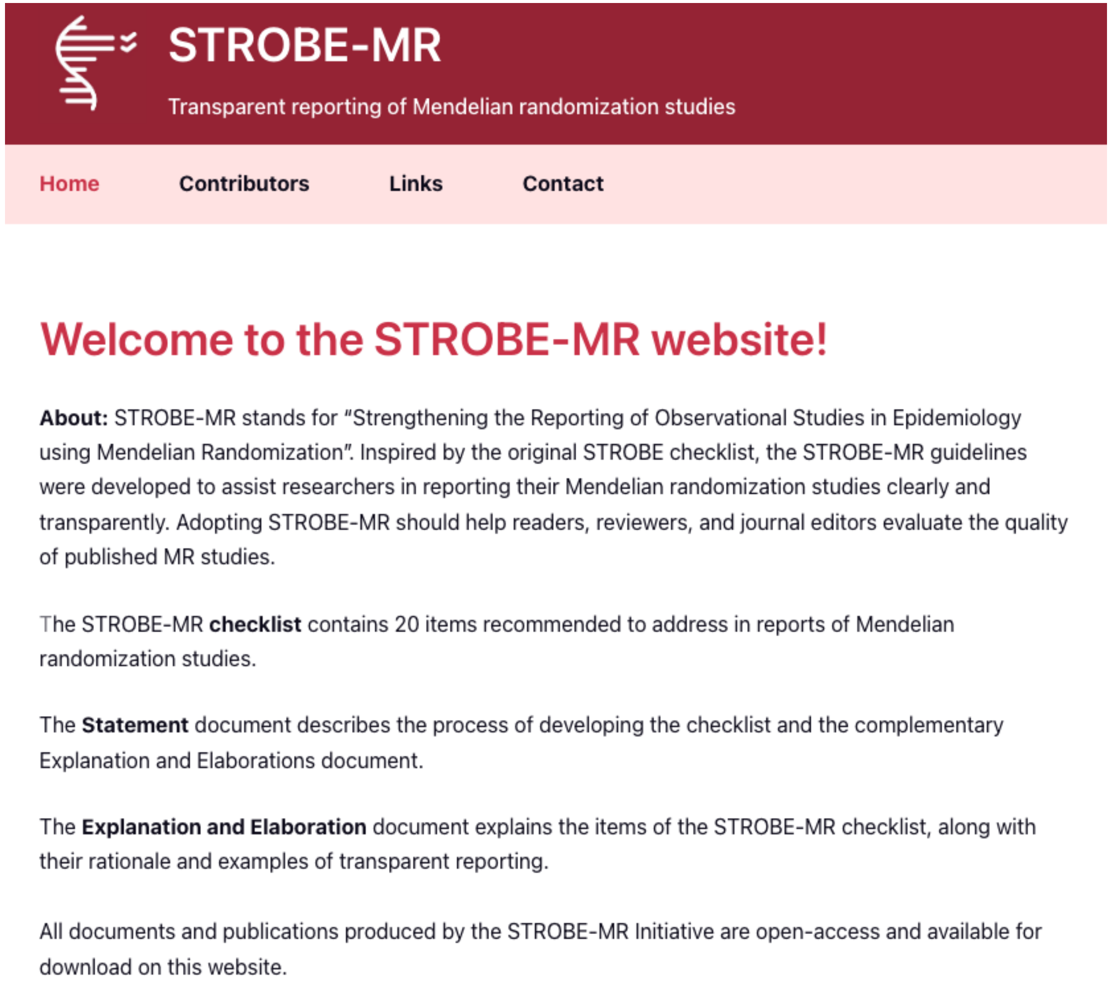
Select methods for analysis

Many methods exist for performing two sample MR. Different methods have sensitivities to different potential issues, accommodate different scenarios, and vary in their statistical efficiency.

Choose which methods to use:

- ☑ Wald ratio
- ☐ Maximum likelihood
- ☑ MR Egger
- ☐ MR Egger (bootstrap)
- ☐ Simple median
- ☑ Weighted median
- ☐ Penalised weighted median
- ☑ Inverse variance weighted
- ☐ IVW radial
- ☐ Inverse variance weighted (multiplicative random effects)
- ☐ Inverse variance weighted (fixed effects)
- ☐ Simple mode
- ☑ Weighted mode
- ☐ Weighted mode (NOME)
- ☐ Simple mode (NOME)
- ☐ Robust adjusted profile score (RAPS)
- ☐ Sign concordance test
- ☐ Unweighted regression

STROBE-MR



Welcome to the STROBE-MR website!

About: STROBE-MR stands for “Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization”. Inspired by the original STROBE checklist, the STROBE-MR guidelines were developed to assist researchers in reporting their Mendelian randomization studies clearly and transparently. Adopting STROBE-MR should help readers, reviewers, and journal editors evaluate the quality of published MR studies.

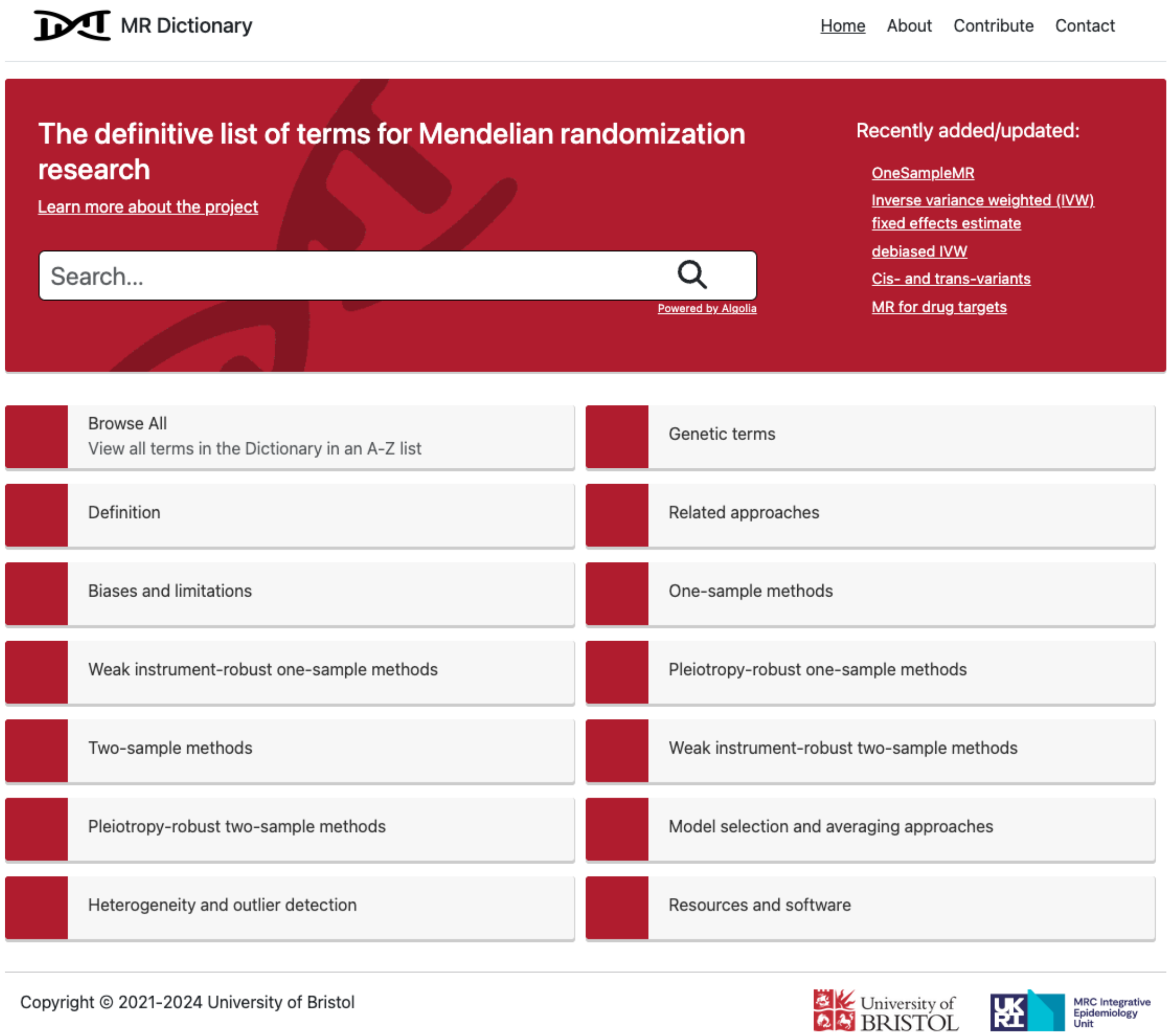
The STROBE-MR **checklist** contains 20 items recommended to address in reports of Mendelian randomization studies.

The **Statement** document describes the process of developing the checklist and the complementary Explanation and Elaborations document.

The **Explanation and Elaboration** document explains the items of the STROBE-MR checklist, along with their rationale and examples of transparent reporting.

All documents and publications produced by the STROBE-MR Initiative are open-access and available for download on this website.

MR Dictionary



The definitive list of terms for Mendelian randomization research

Learn more about the project

Recently added/updated:

- OneSampleMR
- Inverse variance weighted (IVW) fixed effects estimate
- debiased IVW
- Cis- and trans-variants
- MR for drug targets

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 a hypothesized association is not causal

- Horizontal pleiotropy is one of the main threats to the validity of MR studies
  - Multiple methods developed to detect and adjust for horizontal pleiotropy

- Crucial to perform sensitivity analyses and obtain metrics regarding the likely reliability of the MR estimates

- Consistency of results across methods is key to reliable causal inference

Additional References

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