Mendelian Randomization

Using genes to proxy environmental exposures to inform causality

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Outline

Problems with observational data

Randomized controlled trials

Mendelian randomization (MR)

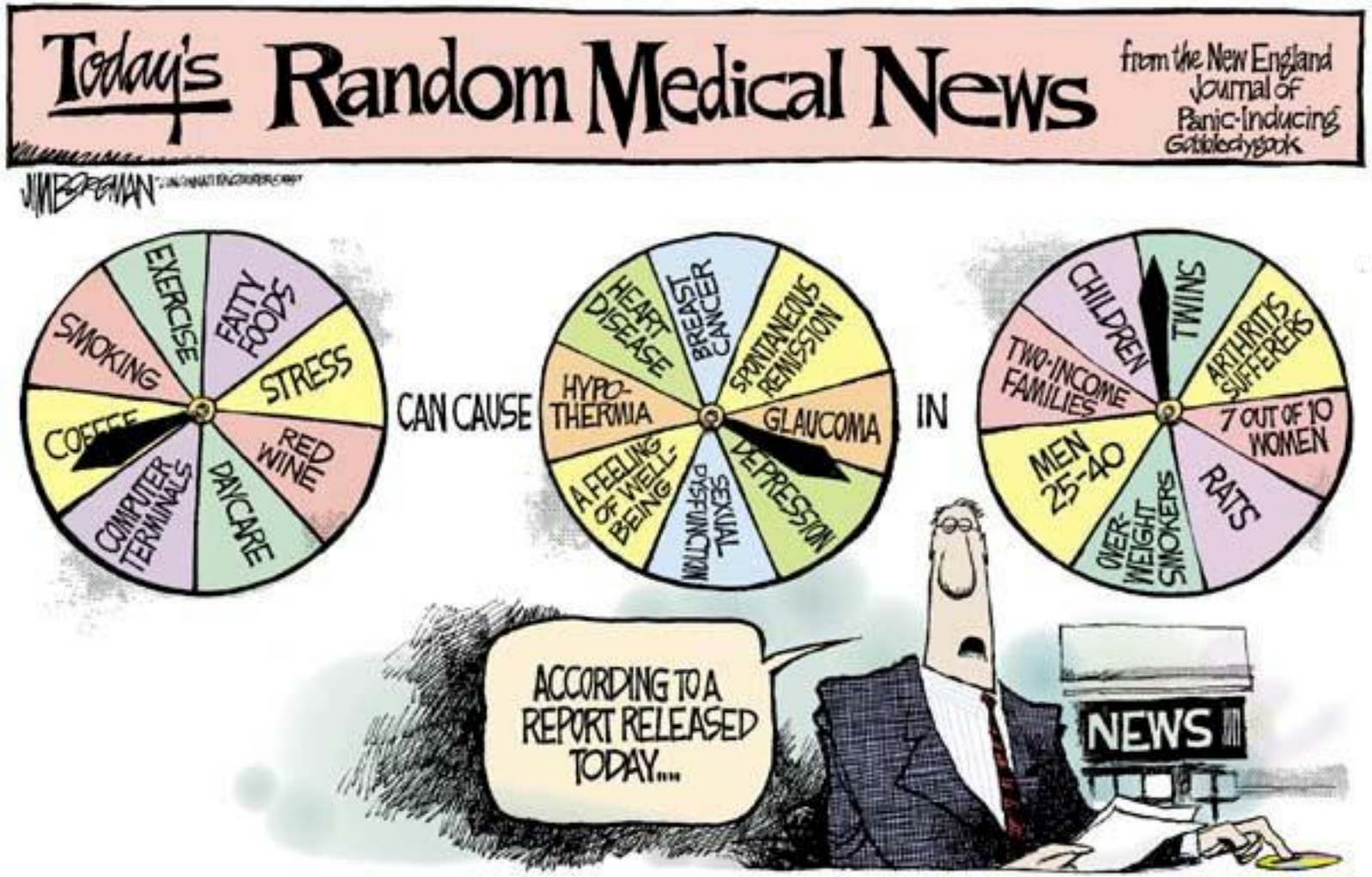
- How it works
- Core assumptions
- Calculating causal effect estimates

MR example

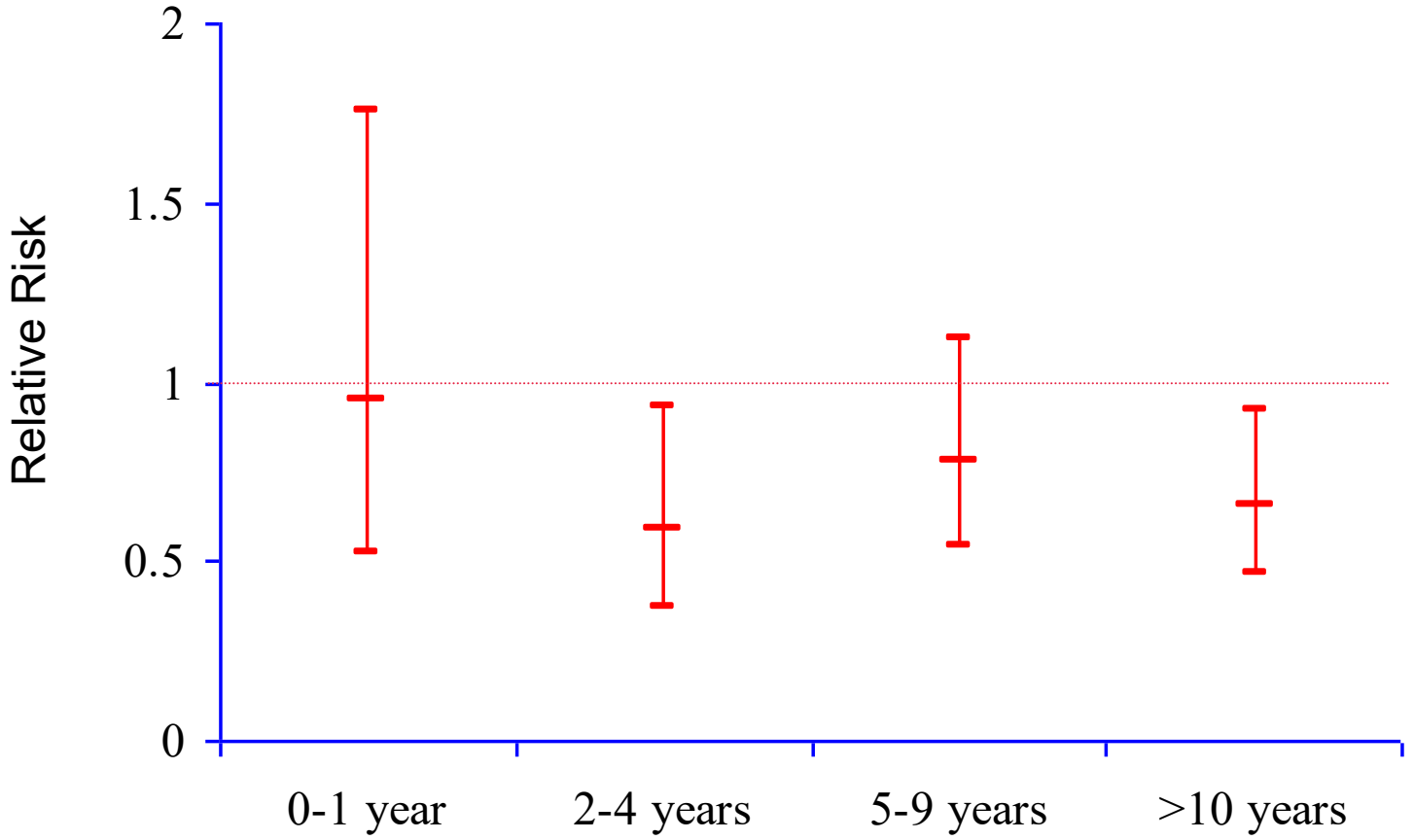
Limitations of MR

Software tools

Problems with inferring causality in observational studies

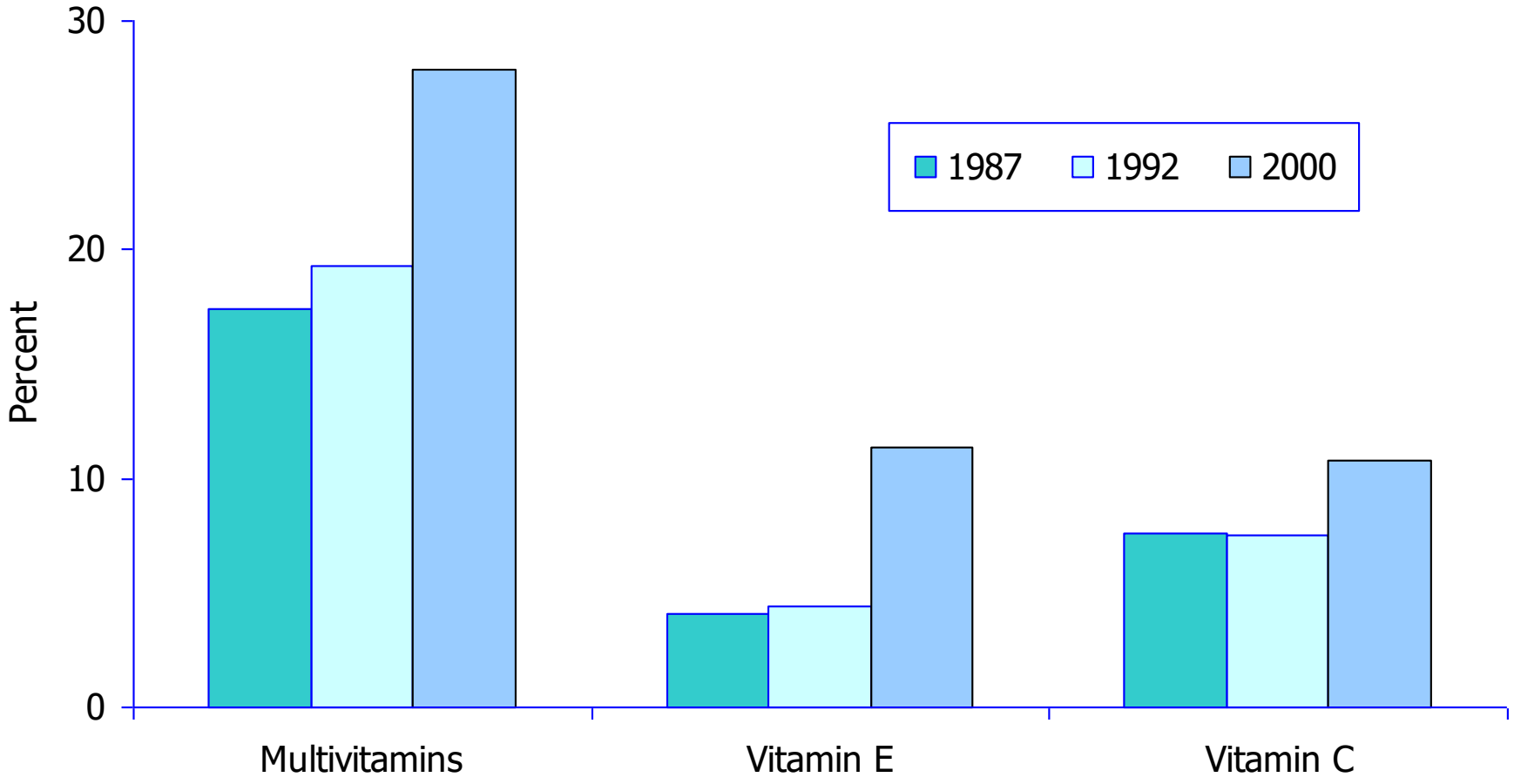


Coronary Heart Disease risk according to duration of current Vitamin E supplement use compared to no use



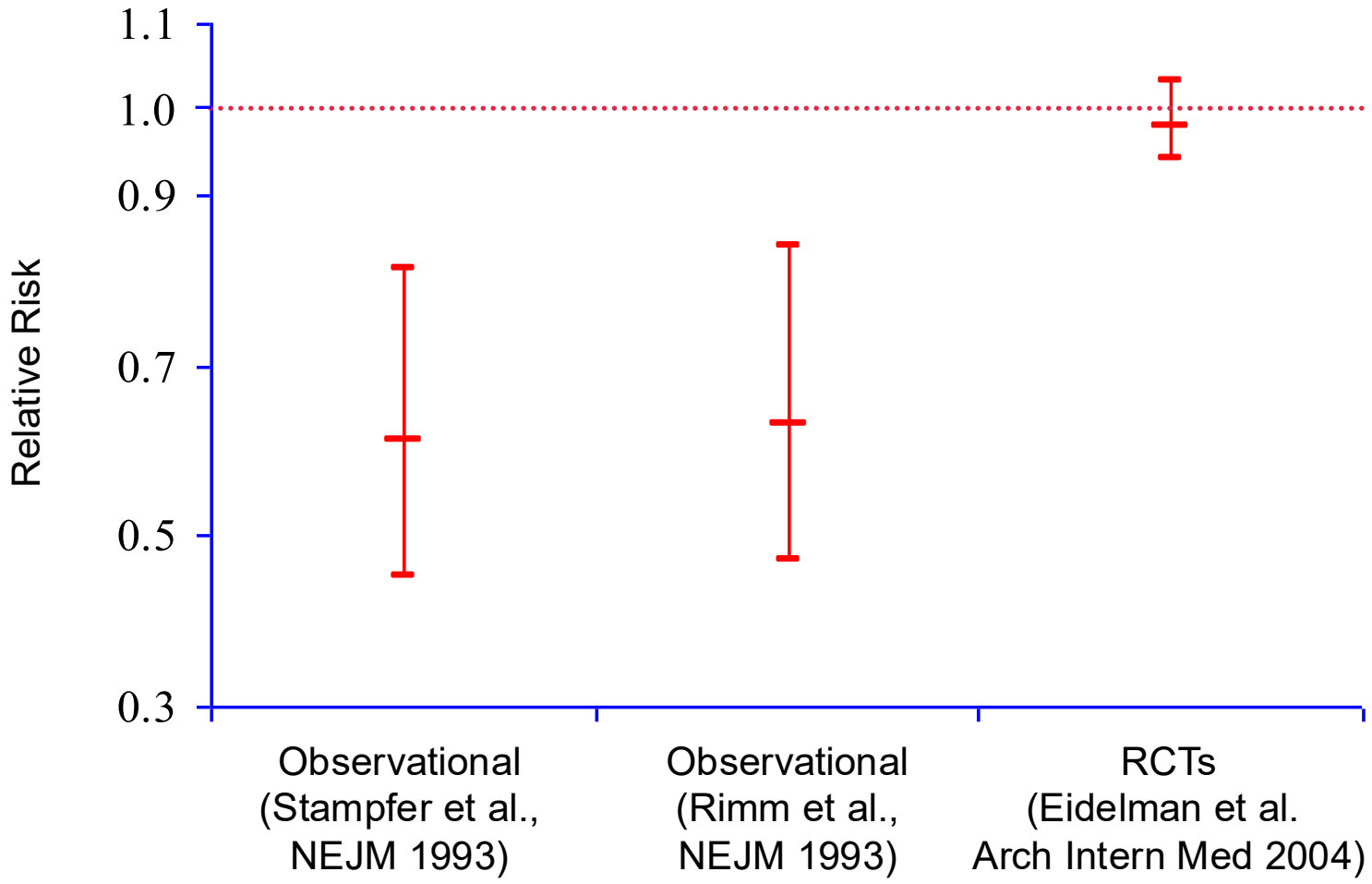
Rimm et al. NEJM 1993; 328: 1450-6

Use of vitamin supplements by US adults



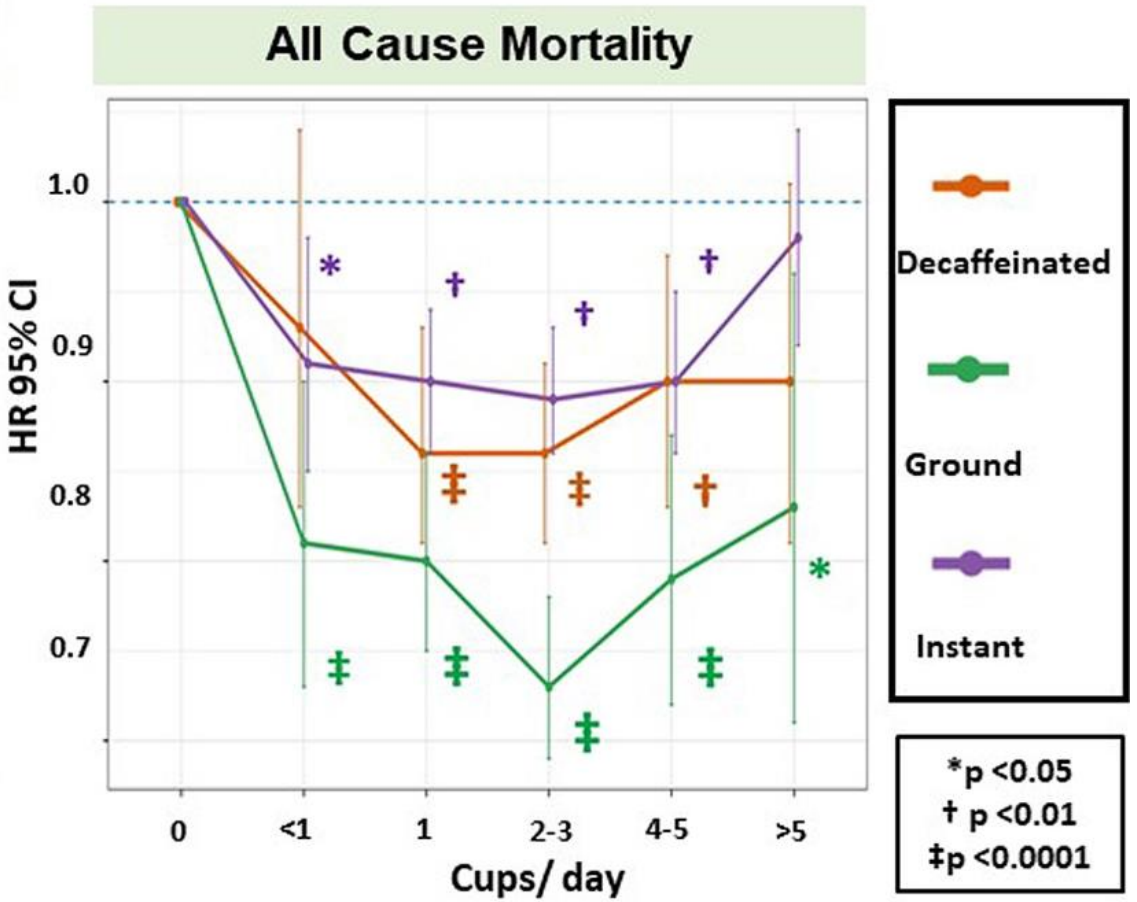
Millen AE, Journal of American Dietetic Assoc 2004;104:942-950

Vitamin E supplement use and risk of Coronary Heart Disease



> Eur J Prev Cardiol. 2022 Dec 7;29(17):2240-2249. doi: 10.1093/eurjpc/zwac189.

The impact of coffee subtypes on incident cardiovascular disease, arrhythmias, and mortality: long-term outcomes from the UK Biobank



"*It turns out that drinking a few cups of coffee each day may actually do more than just give you a jolt at work — **it might even help you live longer***." – CBS News.

"*You've probably heard of the many benefits of drinking coffee…But did you know that **coffee can also increase your lifespan**? That's what scientists behind a new research study announced recently*." – USA Today.

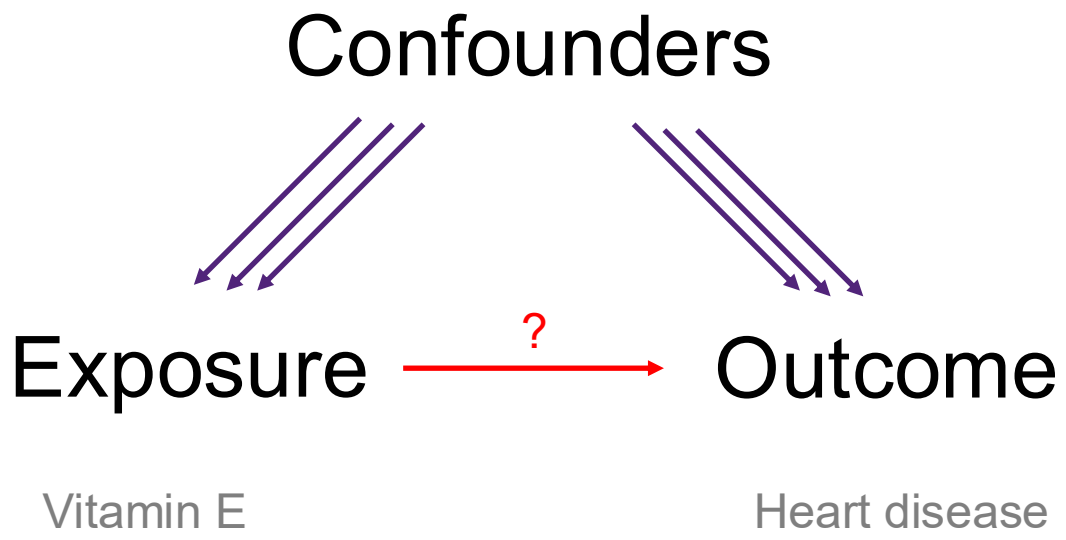
Many other examples

Vitamin C, vitamin A, hormone replacement therapy (HRT), many drug targets…

What's the explanation for these observational associations?

Confounding

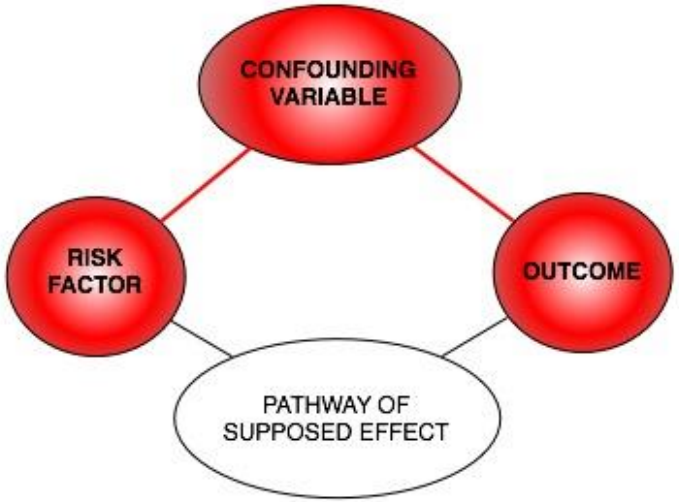
Smoking, diet, alcohol, socioeconomic position….



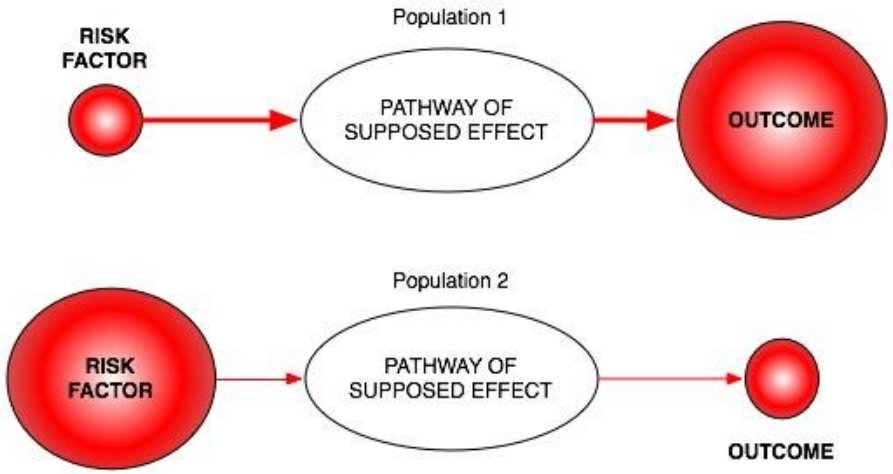
Classic limitations to "observational" science

Reverse Causation

Confounding

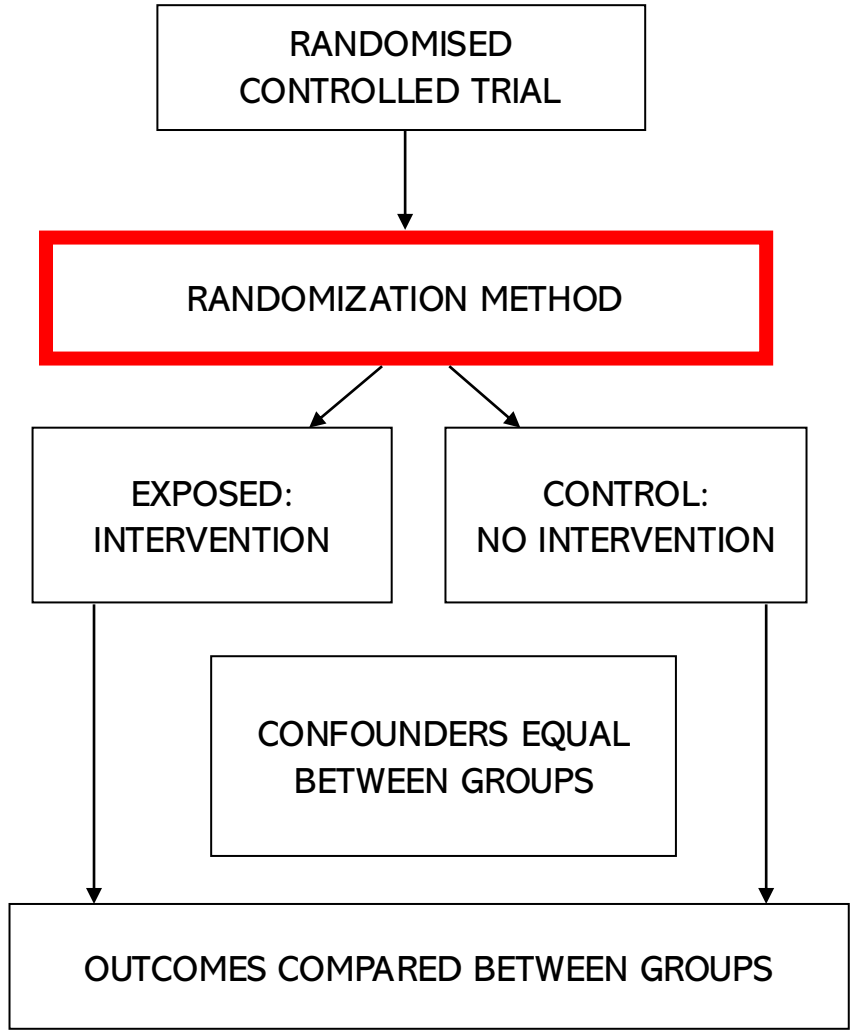


Bias



Randomized Controlled Trials (RCTs)
The Gold Standard in Inferring Causality

Randomization makes causal inference possible



The Need for Observational Studies

Randomized Controlled Trials (RCTs):

- Not always ethical or practically feasible, e.g. anything toxic
- Expensive, requires experimentation in humans, and long follow-up times
- Should only be conducted on interventions that show very strong observational evidence in humans

Observational studies:

- Association between environmental exposures and disease measured in observational designs (non-experimental)
  - e.g. case-control studies or cohort studies
- Reliably assigning causality in these types of studies is ***very limited***

Mendelian Randomization (MR)

A technique that uses genetically informative observational data to inform causality

What does MR do?

1. Assess causal relationship between two variables
   - Useful in genomics studies
2. Estimate magnitude of causal effect
   - Useful in drug development
   - Useful in public health

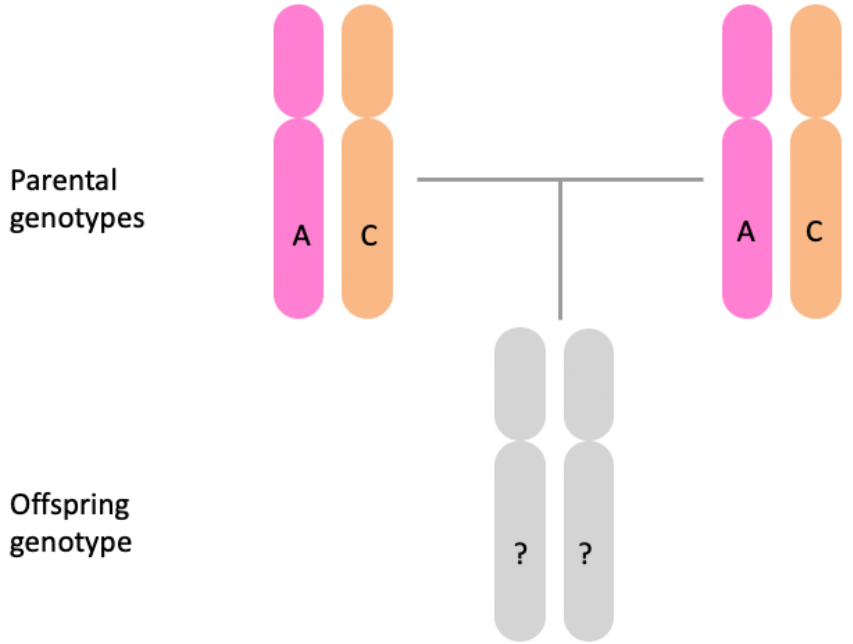
How does MR do this?

By harnessing Mendel's laws of inheritance

Mendel's Laws of Inheritance

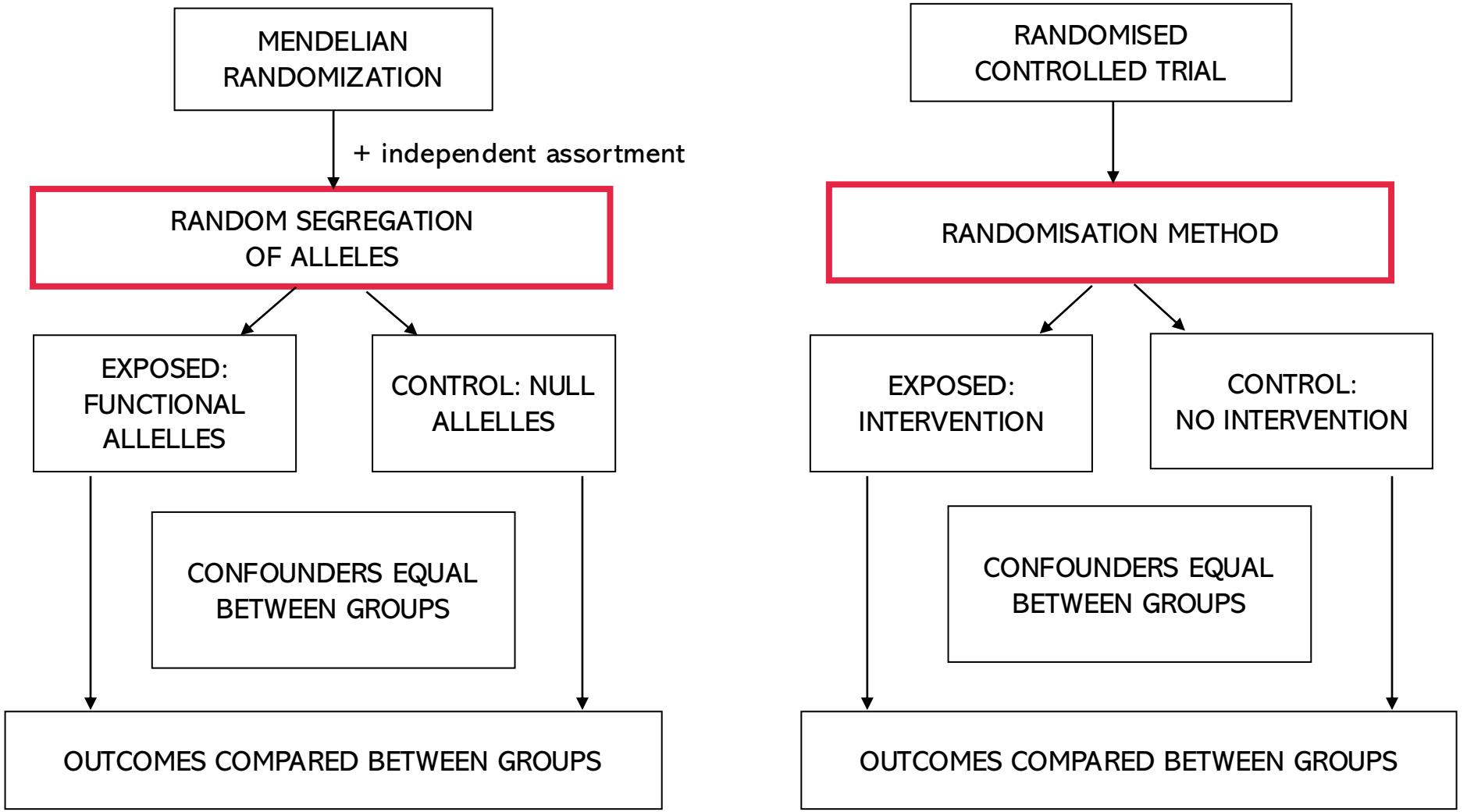
Gregor Mendel in 1862

1. **Segregation:** when alleles separate at meiosis, a <u>randomly selected allele</u> is transmitted to offspring

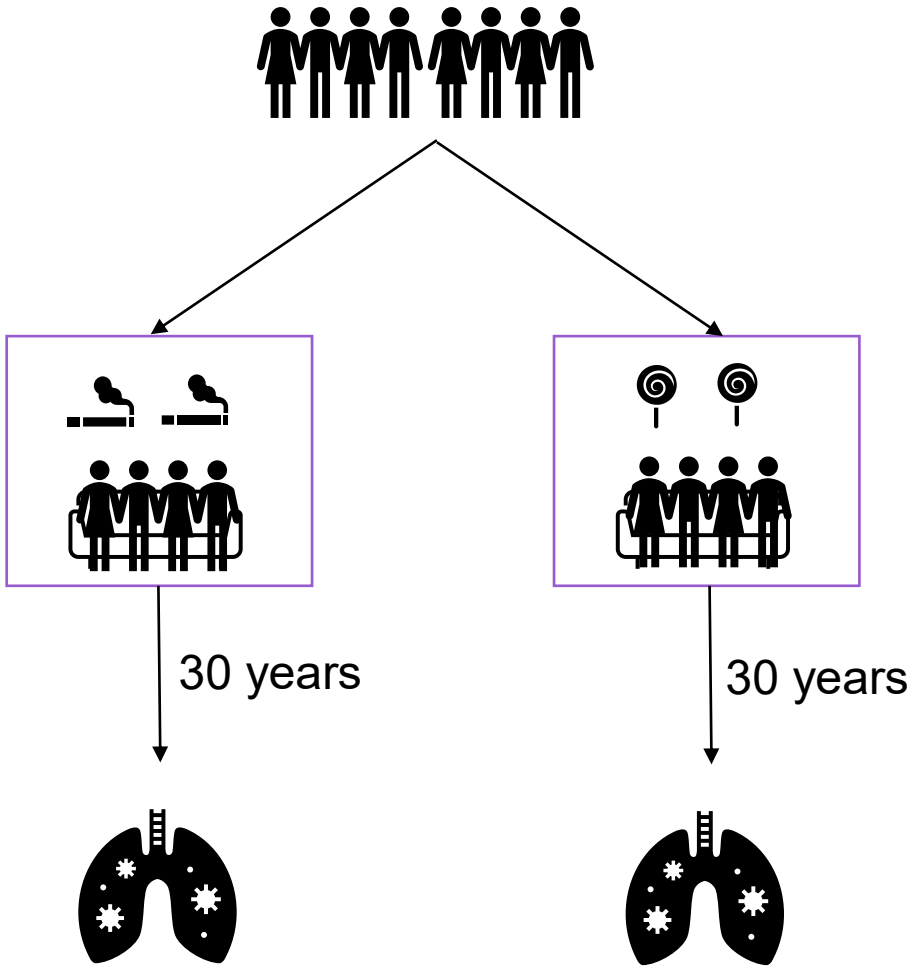


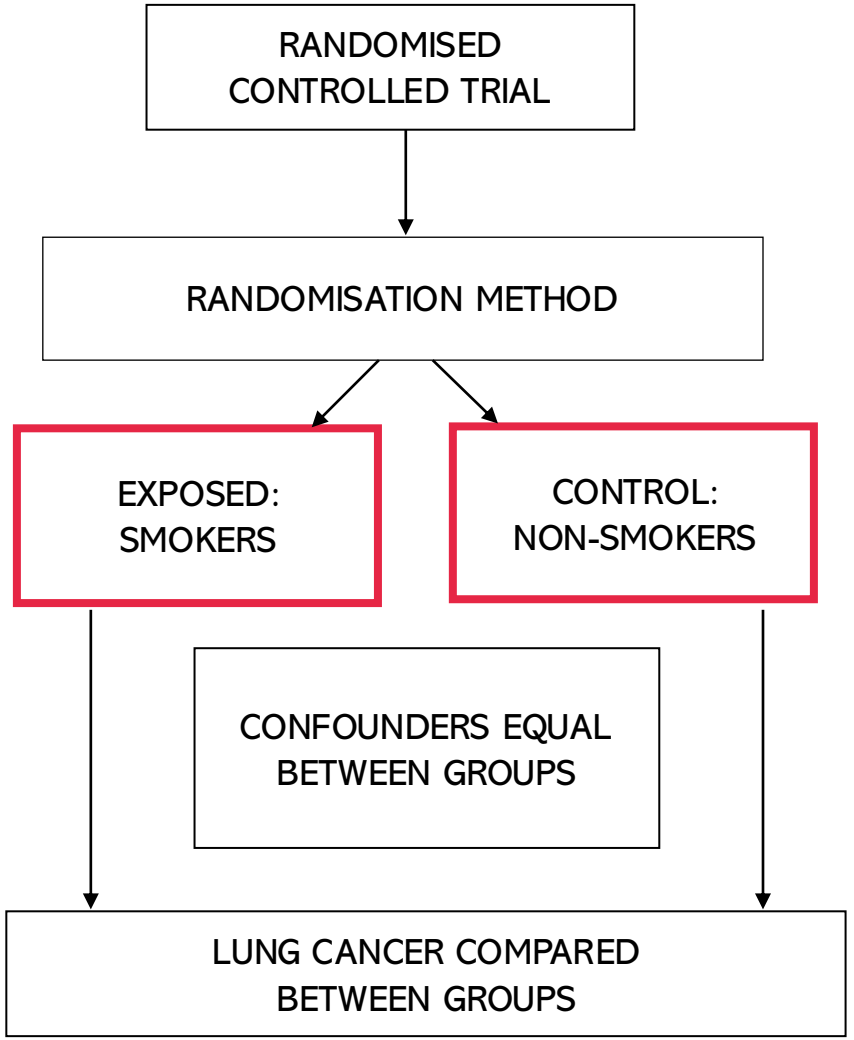
2. **Independent assortment:** alleles at different genetic loci (for different traits) are transmitted <u>independently</u> of one another

MR vs RCT

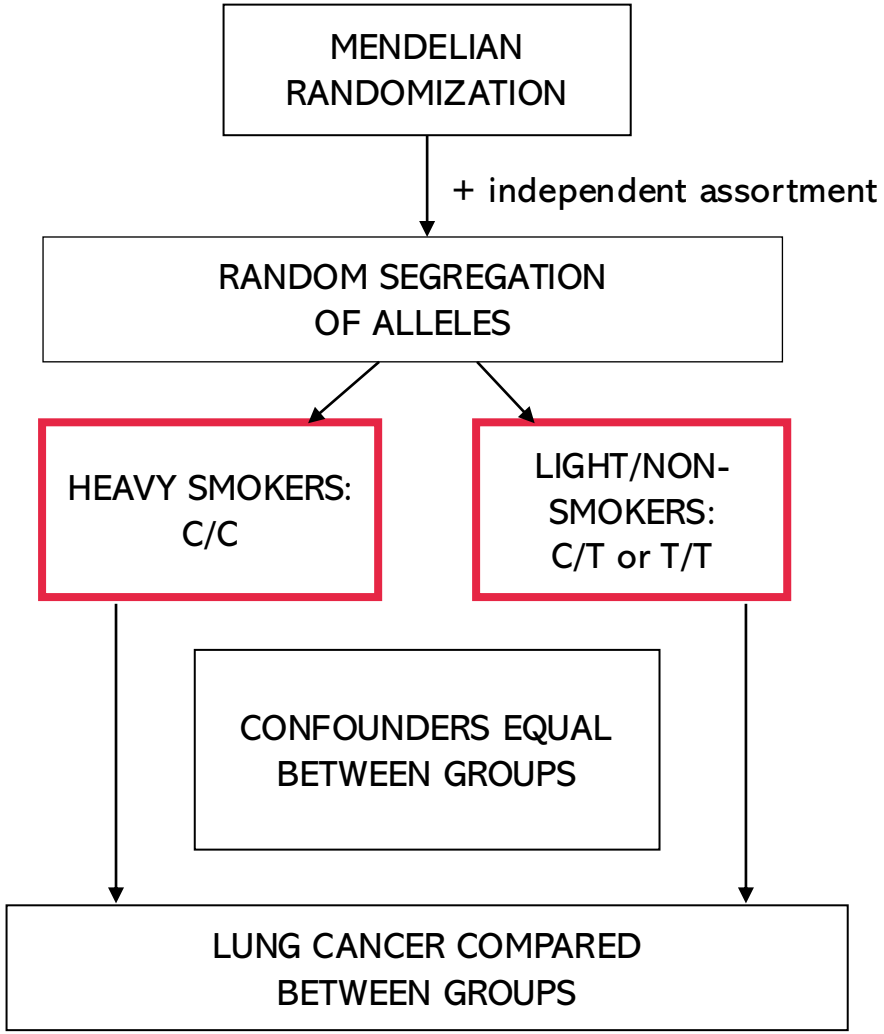

MENDELIAN RANDOMIZATION
+ independent assortment
RANDOM SEGREGATION OF ALLELES
EXPOSED: FUNCTIONAL ALLELLES
CONTROL: NULL ALLELLES
CONFOUNDERS EQUAL BETWEEN GROUPS
OUTCOMES COMPARED BETWEEN GROUPS
RANDOMISED CONTROLLED TRIAL
RANDOMISATION METHOD
EXPOSED: INTERVENTION
CONTROL: NO INTERVENTION
CONFOUNDERS EQUAL BETWEEN GROUPS
OUTCOMES COMPARED BETWEEN GROUPS

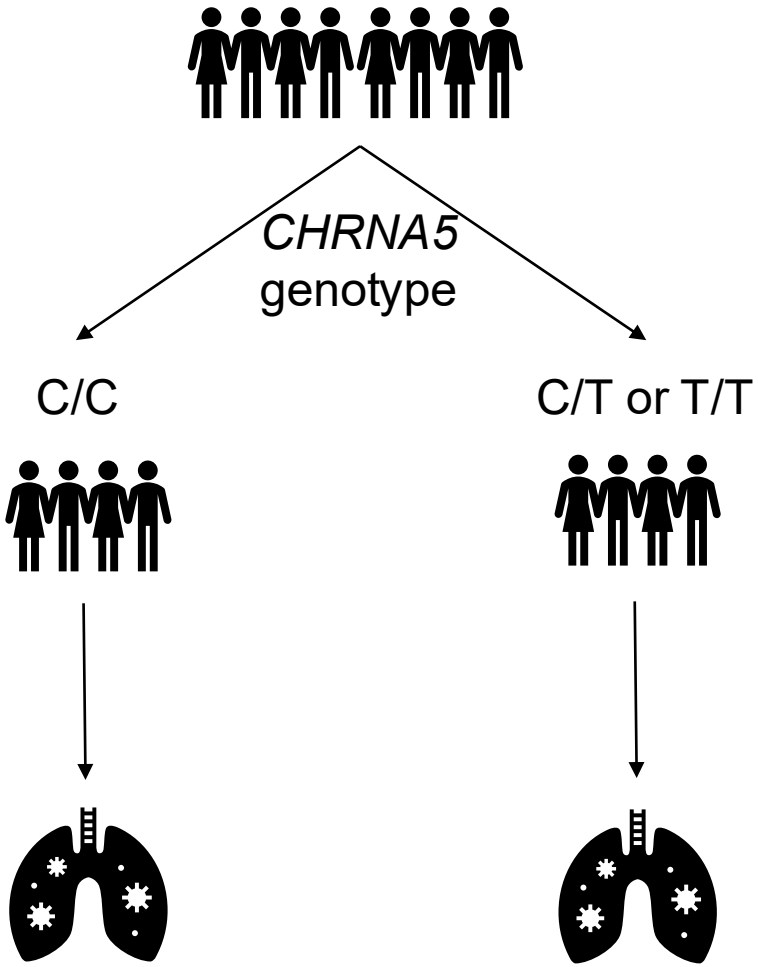
RCT: Smoking and Lung Cancer



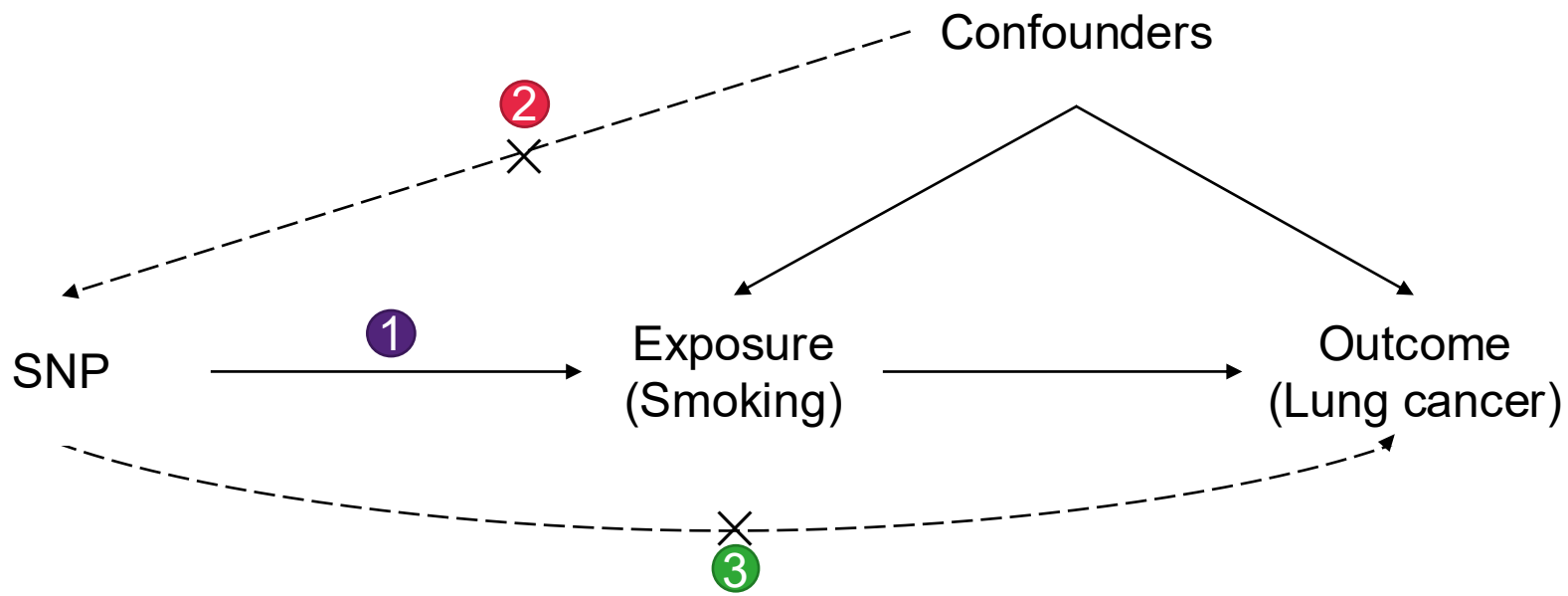


MR: Smoking and Lung Cancer





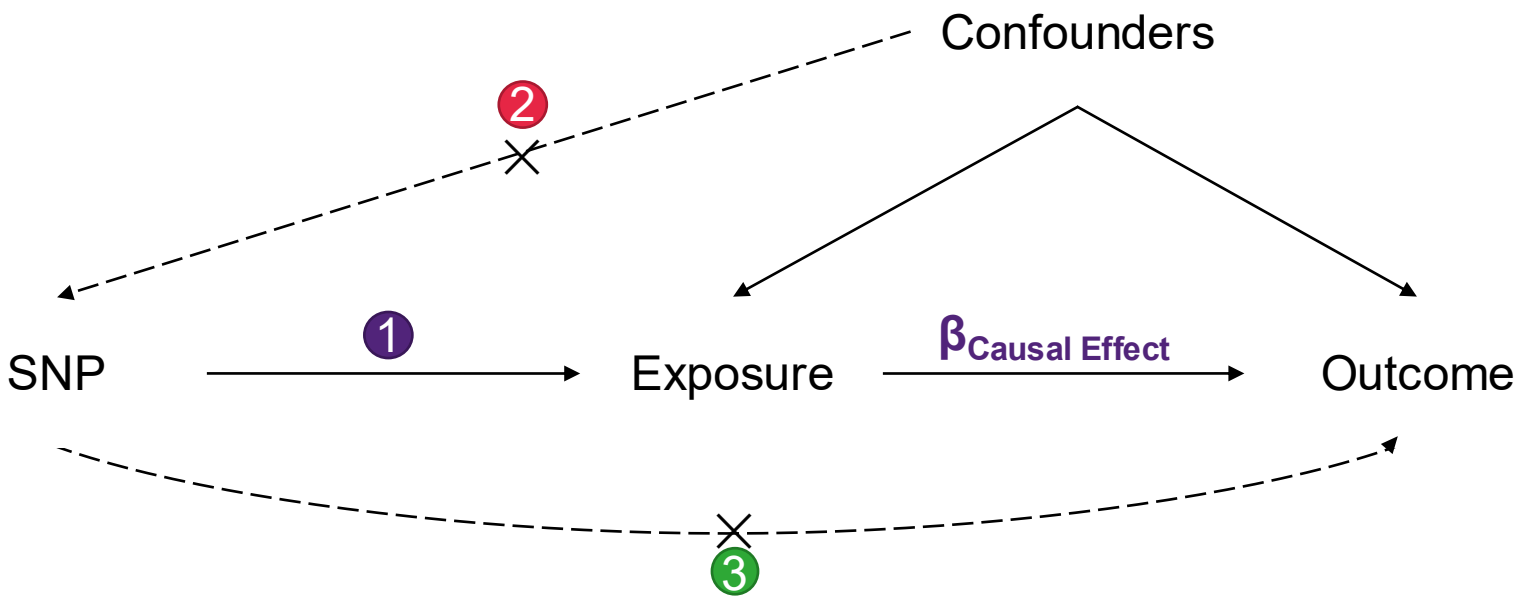
Mendelian Randomization: 3 Core Assumptions



(1) Relevance assumption: SNP is robustly associated with the exposure
(2) Independence assumption: There are no confounders of the association between the instrumental variables (IVs) and the outcome.
(3) Exclusion restriction: SNP is ONLY associated with the outcome through the exposure

SNPs are identified as **good instrumental variables** when the 3 assumptions are met!

Calculating Causal Effect Estimates



Two common approaches to calculate causal effects using a single SNP:

- Two-stage least-squares (TSLS) regression
- Wald Estimator

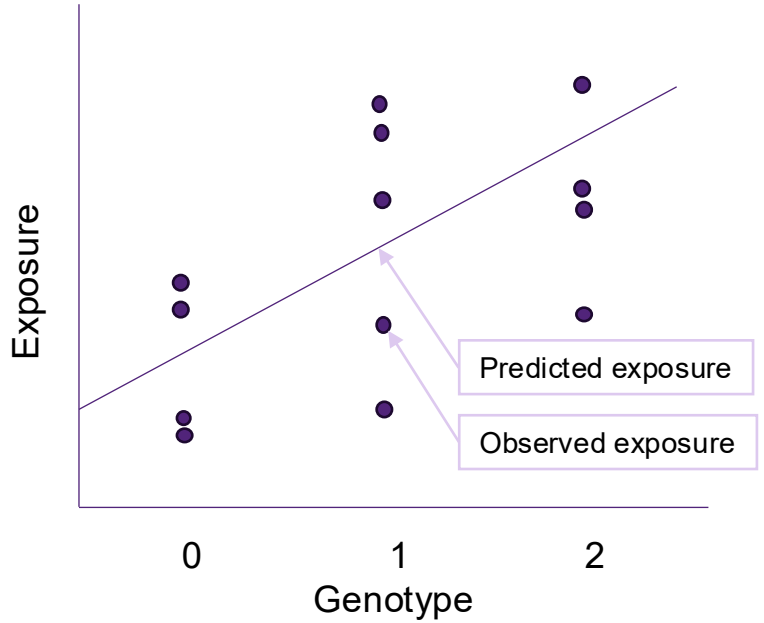
Calculating Causal Effect Estimates: Two-Stage Least Squares

A single sample of individuals with data on the SNP, the exposure and the outcome. Also known as “One sample MR”.

Manual calculation:

1. Regress exposure on SNP and obtain predicted values
2. Regress outcome on **predicted** exposure (from 1st stage regression)

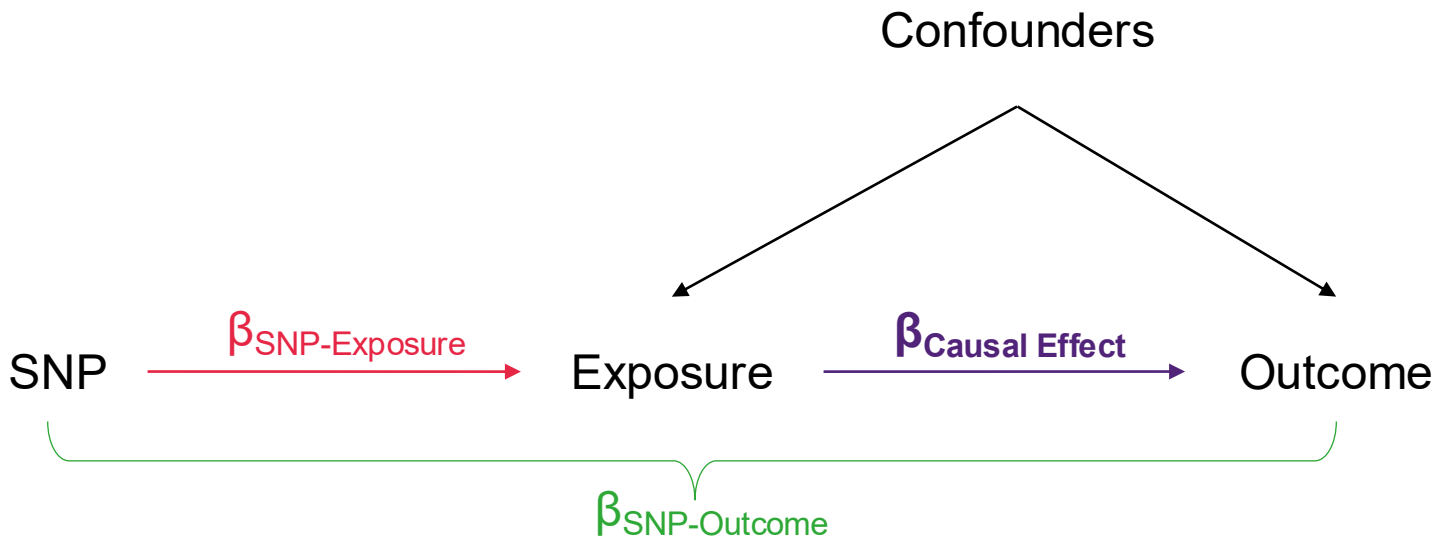
The regression coefficient from the second stage is the estimate of the causal effect of the exposure on the outcome.



This gives you: difference in outcome per unit change in (genetically-predicted) exposure

Genetically determined exposure → “randomized” → can ascribe causality
(if assumptions are met)

Calculating Causal Effect Estimates
Wald Estimator (Wald Ratio)



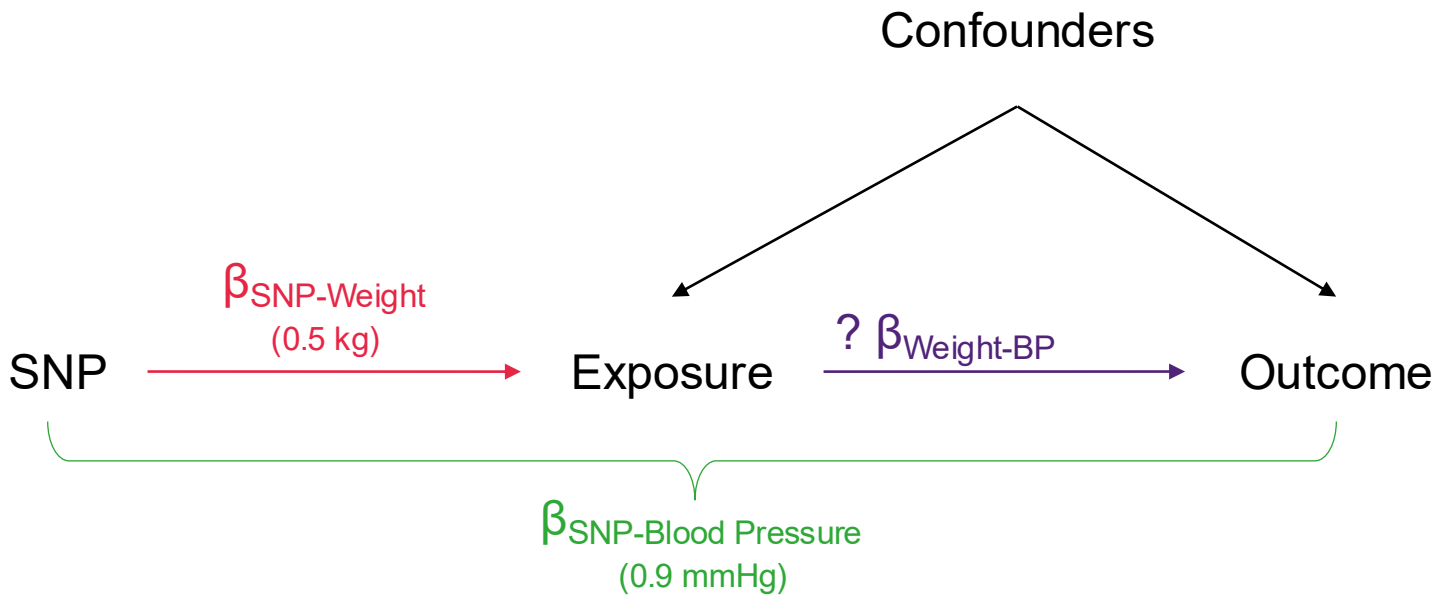
When there is a linear relationship between SNP, exposure and outcome:

$\beta_{SNP\text{-}Outcome} = \beta_{Causal\ Effect} \times \beta_{SNP\text{-}Exposure}$

$$\beta_{Causal\ Effect}\ (\text{Wald estimator}) = \frac{\beta_{SNP\text{-}Outcome}}{\beta_{SNP\text{-}Exposure}}$$

(change in outcome per unit change in exposure)

Calculating Causal Effect Estimates
Wald Estimator (Wald Ratio)



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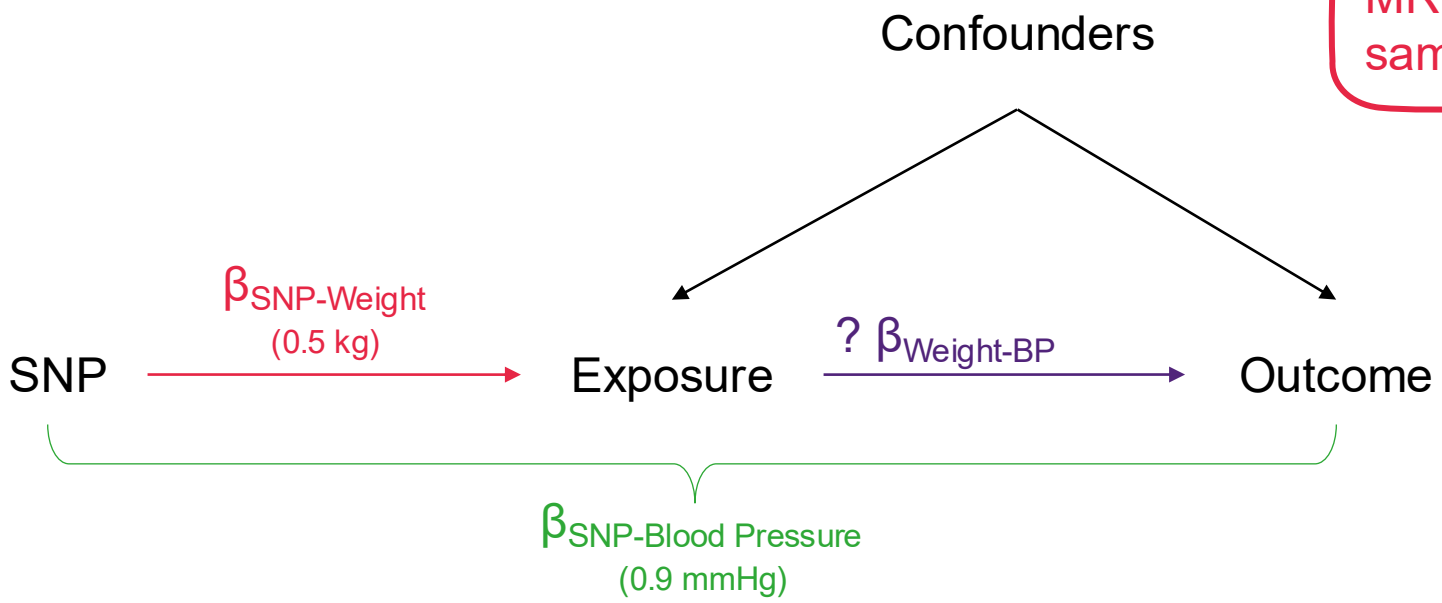
$$\beta_{Weight\text{-}BP} = \frac{0.9\ \text{mmHg/allele}}{0.5\ \text{kg/allele}} = 1.8\ \text{mmHg/kg}$$

Calculating Causal Effect Estimates
Wald Estimator (Wald Ratio)

Wald estimator can be used in one sample ("One sample MR") as well as different samples ("Two sample MR")



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MR can also be performed using just the results from GWAS

Also known as “two-sample MR”, SMR, or MR with summary data etc

- SNP-exposure associations obtained from GWAS of sample 1
- SNP-outcome associations from GWAS of sample 2

Advantages:

- The data is readily available, non-disclosive, free, open source
- The exposure and outcome might not be measured in the same sample
- The sample size of the outcome variable, key to statistical power, is not limited by requiring overlapping measures of the exposure

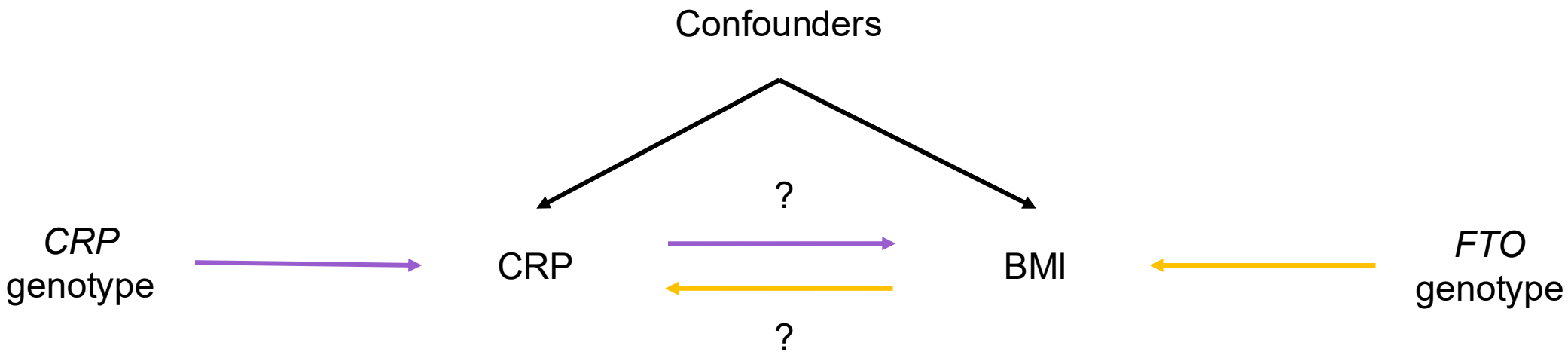
Disadvantages:

- Some extensions of MR not possible, e.g. non-linear MR, use of GxE for negative controls, various sensitivity analyses

MR Example using CRP and BMI

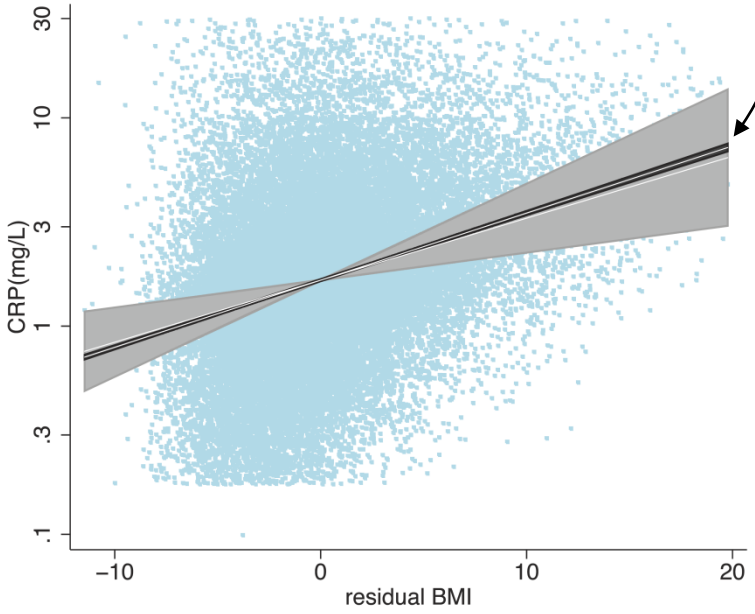
- C-Reactive Protein (CRP) is a biomarker of inflammation
- It is associated with BMI, metabolic syndrome, CHD and a number of other diseases
- It is unclear whether these observational relationships are causal or due to confounding or reverse causality
- This question is important from the perspective of intervention and drug development

“Bi-directional Mendelian Randomization”
Testing causality and reverse causation



“Bi-directional Mendelian Randomization”
Testing causality and reverse causation

	Effect estimates				
Exposure → Outcome	Observational association	Instrumental variable (MR)	P_{IV}	P_{diff}	F_{first}
BMI → CRP	1.075 (1.073, 1.077)	1.06 (1.02, 1.11)	0.002	0.6	50.2



Timpson NJ et al. *Int J Obes* (2011) 35(2):300-308

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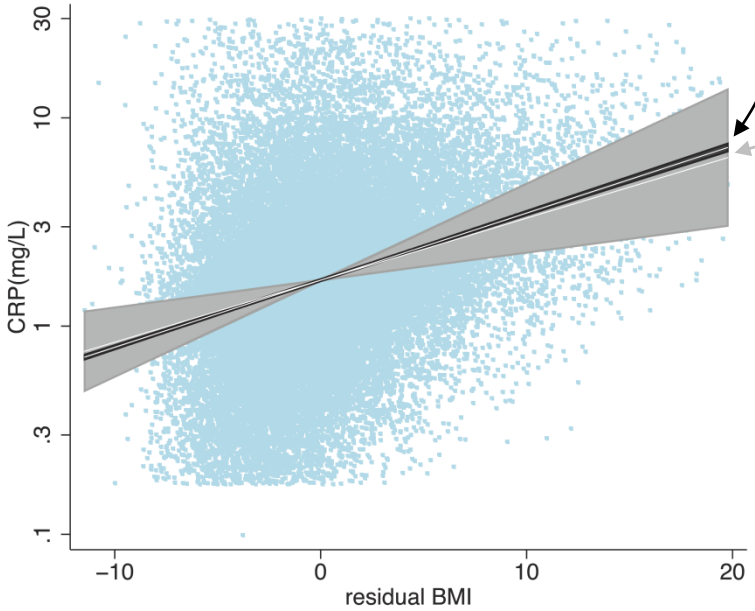

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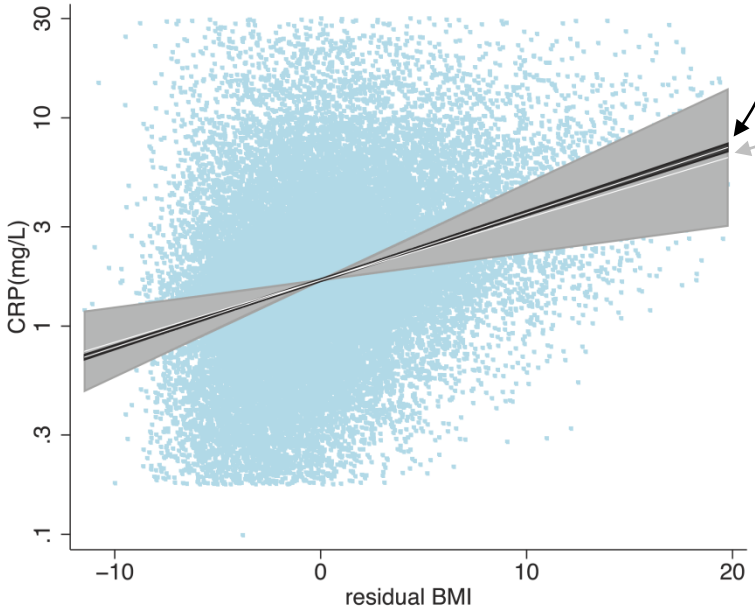

P_{diff} is calculated by conducting a heterogeneity test of the effect estimates from the observational association and instrumental variable analysis.

“Bi-directional Mendelian Randomization”
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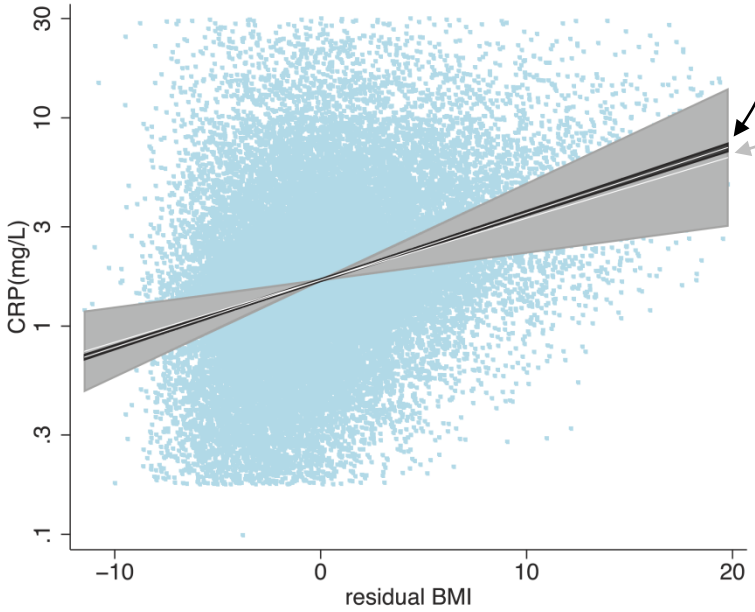


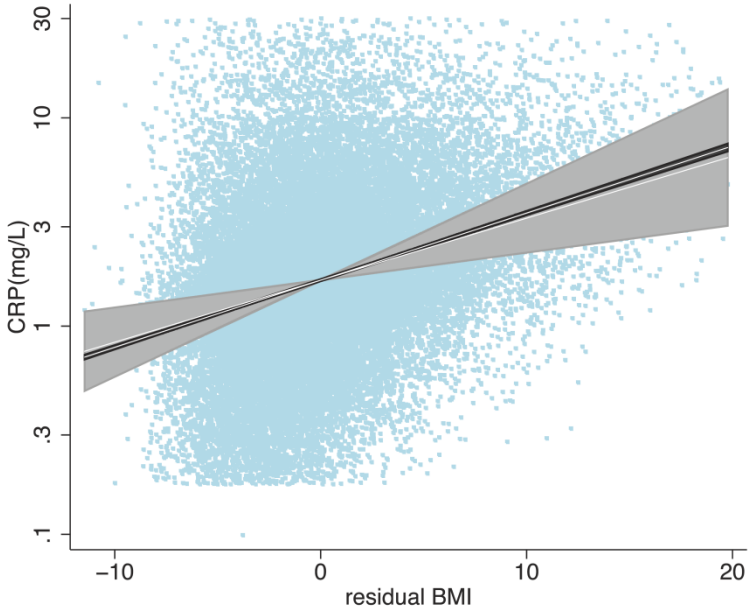
P_{diff} is calculated by conducting a heterogeneity test of the effect estimates from the observational association and instrumental variable analysis.

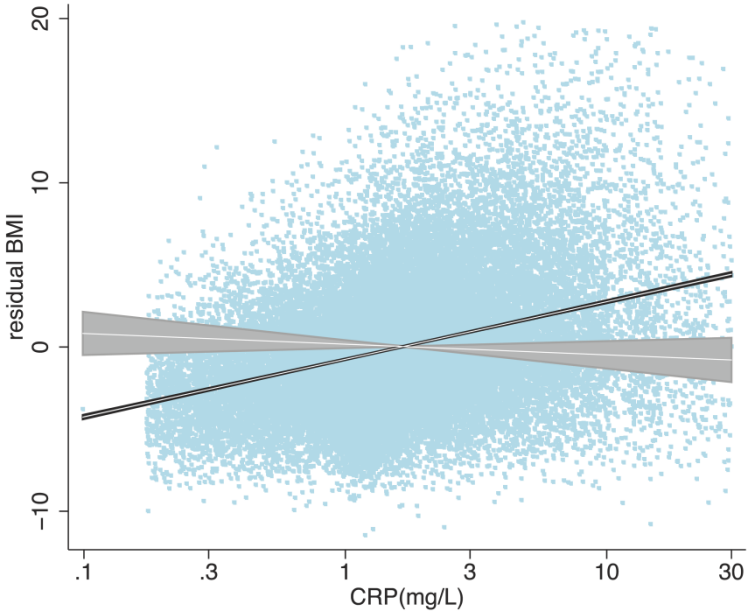
$$F_{first} = \frac{R^2 * (N-1)}{(1-R^2)}$$

R^2 is the variance explained in exposure by the SNP(s)
N is number of individuals in the study.

“Bi-directional Mendelian Randomization”
Testing causality and reverse causation

	Effect estimates				
Exposure → Outcome	Observational association	Instrumental variable (MR)	P_{IV}	P_{diff}	F_{first}
BMI → CRP ✓	1.075 (1.073, 1.077)	1.06 (1.02, 1.11)	0.002	0.6	50.2
CRP → BMI	0.58 (1.53, 1.63)	-0.30 (-0.78, 0.18)	0.2	<0.00001	78.3





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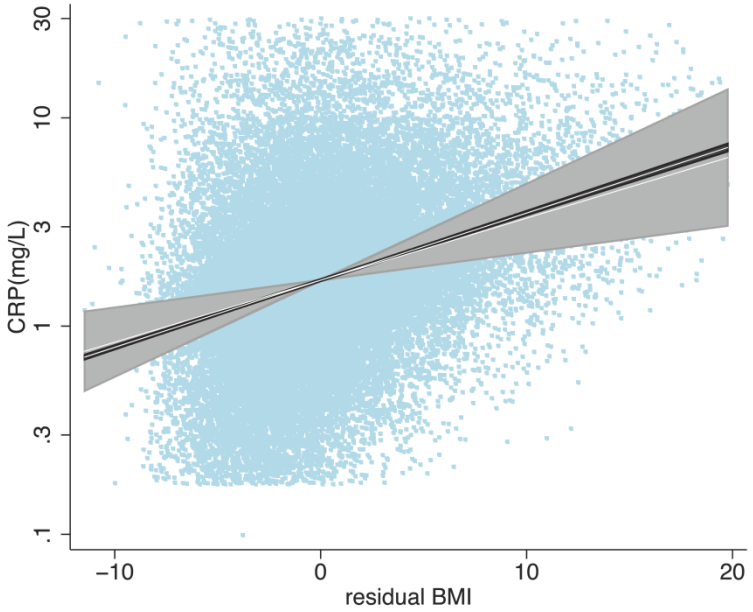
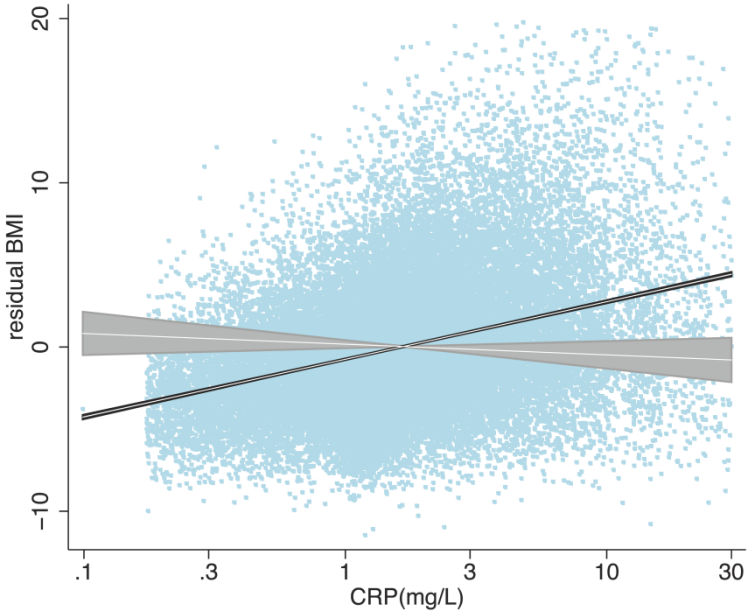




Limitations to Mendelian Randomization

1. Population stratification
2. Canalization (“Developmental compensation”)
3. The existence of instruments
4. **Power (also “weak instrument bias”)**
5. **Pleiotropy**

Power and Weak Instruments

Power:

- Genetic variants explain very small amounts of phenotypic variance in a given trait
- VERY large sample sizes are generally required to demonstrate causal effects

Weak instruments (weak instrument bias):

- Genetic variants that are weak proxies for the exposure
- Results in biased causal estimates from MR
  - **Single Sample MR:** to the confounded estimate
  - **Two-Sample MR:** to the null

Using Multiple Genetic Variants as Instruments

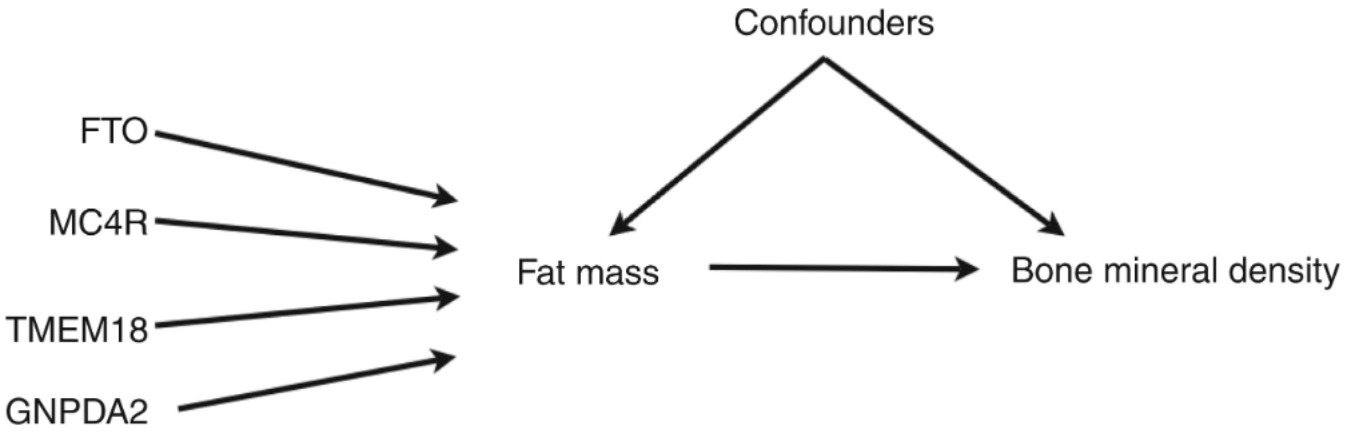


Figure 1. DAG for a Mendelian randomisation analysis using four genetic variants as instrumental variables for the effect of fat mass on bone mineral density.

- Creating allelic scores using multiple genetic variants
- Testing multiple variants individually and then meta-analysing individual SNPs

Palmer et al (2011) Stat Method Res

Calculating Power in Mendelian Randomization Studies (https://shiny.cnsgenomics.com/mRnd/)

mRnd: Power calculations for Mendelian Randomization

Input

Calculate:

- (●) Power
- ( ) Sample size

Provide:

Sample size

1000

α

0.05

Type-I error rate

β_{yx}

0

The regression coefficient β_{yx} for the true underlying causal association between the exposure (X) and outcome (Y) variables

β_{OLS}

0

The regression coefficient β_{OLS} for the observational association between the exposure (X) and outcome (Y) variables

R^2_{xz}

0.01

Proportion of variance explained for the association between the SNP or allele score (Z) and the exposure variable (X)

$\sigma^2(x)$

1

Variance of the exposure variable (X)

Continuous outcome | Binary outcome | Binary outcome derivations | Citation | About

Two-stage least squares

Power	0.05	
NCP	0.00	Non-Centrality-Parameter
F-statistic	11.10	The strength of the instrument

Power or sample size calculations for two-stage least squares Mendelian Randomization studies using a genetic instrument Z (a SNP or allele score), a continuous exposure variable X (e.g. body mass index [BMI, $\frac{kg}{m^2}$]) and a continuous outcome variable Y (e.g. blood pressure [mmHg]).

YZ association

Power	0.05	
NCP	0.00	Non-Centrality-Parameter

Power or sample size calculations for the regression association of a genetic instrument Z (e.g. a BMI SNP), with a continuous outcome variable Y (blood pressure).

Working Example

If we are interested in calculating the minimum required sample size for performing a Mendelian Randomization (MR) study ascertaining the causal effects of body mass index (BMI) on systolic blood pressure (SBP) in children, the required parameters for this online calculator could be taken from, for example, results from a published observational epidemiology study reporting associations between BMI and SBP and a SNP instrument that is reliably associated with BMI.

In an observational study reporting the association of BMI and SBP in children[1], the regression coefficients for the association between BMI and SBP (averaged coefficients for boys and girls) was observed to be $1.41\frac{mmHg}{SD}$ (no confounder-adjustment) and $1.30\frac{mmHg}{SD}$ [*] (adjusted for confounders). The SD for SBP in this sample (from the paper's online supplementary data) was 10.8, with an SD (standard deviation) of 1 for BMI.

Assume that the causal effect of BMI on SBP is $1.30\frac{mmHg}{SD}$ [*] and that the population regression coefficient of BMI on SBP, including the effects of confounders, is $1.41\frac{mmHg}{SD}$. Also assume that for the MR study we have a genetic instrument that explains $R^2_{xz} = 0.01$ of variation in BMI (based on e.g. FTO SNP, which explains $\sim 1\%$ of the variation in BMI)[2]. Then we can calculate the power of an MR study using the following parameters:

$\beta_{OLS} = 1.41\frac{mmHg}{SD}$

$\beta_{yx} = 1.3\frac{mmHg}{SD}$ [*]

$\sigma^2(x) = 1$

$\sigma^2(y) = 10.8^2 = 116.6\ mmHg^2$

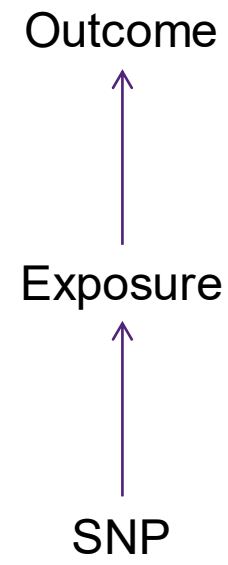
For an α of 0.05 and power of 0.8, the calculated minimum sample size for the Mendelian Randomization study is $N = 53,218$. The reason why this sample size is so large is because BMI explains a small amount of variation in SBP in this case and because the genetic instrument explains a small proportion of variance in BMI.

$*\ \beta_{yx}$ refers to the unknown true causal association between X and Y (between BMI and blood pressure, in this example) and therefore instead of 1.3 mmHg one could potentially use any value of β_{yx} deemed plausible or, for example, inspect the power/sample size calculations for a range of hypothetical values of β_{yx}.

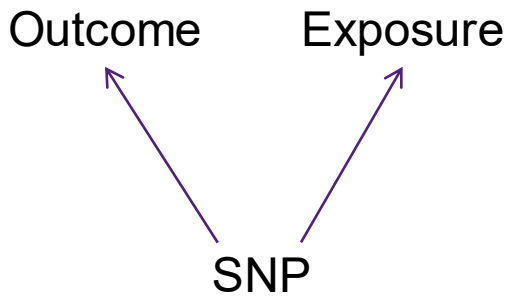
1. Lawlor DA, Benfield L, Logue J et al. Association between general and central adiposity in childhood, and change in these, with cardiovascular risk factors in adolescence: prospective cohort study. BMJ 2010; 341: c6224.

2. Frayling TM, Timpson NJ, Weedon MN et al. A Common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007; 316(5826): 889-894.

Pleiotropy: Genetic variant influences more than one trait



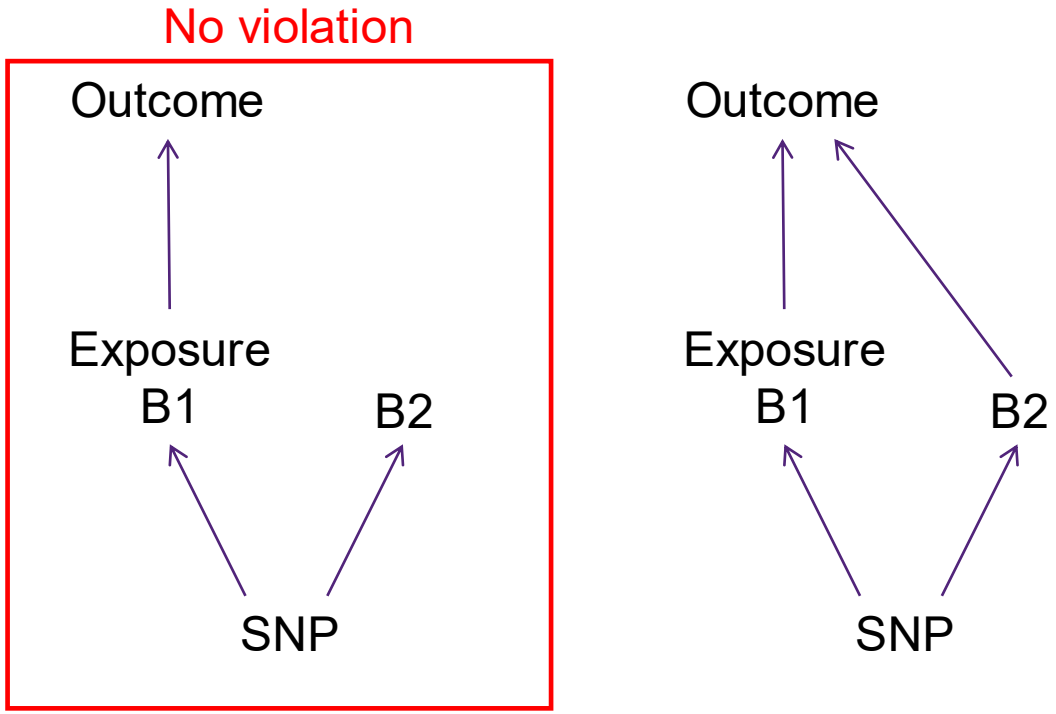
Vertical pleiotropy



Horizontal pleiotropy

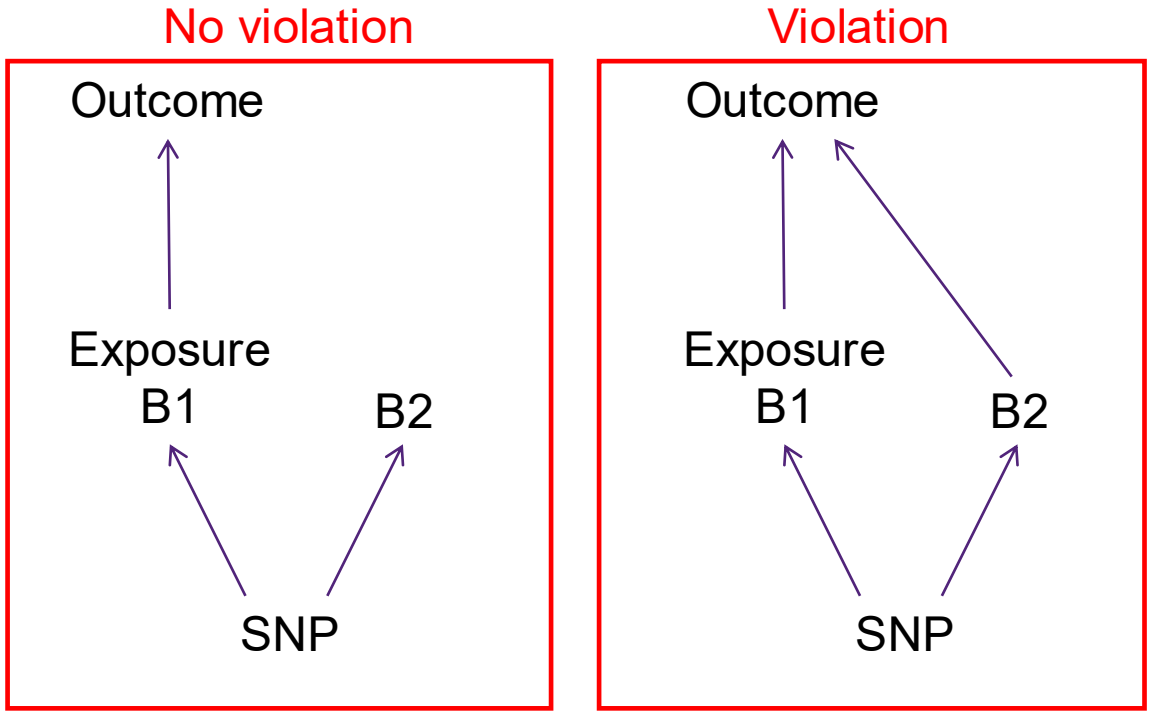
Horizontal Pleiotropy

Pleiotropy only violates MR's assumptions if it involves a pathway outside that of the exposure and is a pathway that affects your outcome.



Horizontal Pleiotropy

Pleiotropy only violates MR's assumptions if it involves a pathway outside that of the exposure and is a pathway that affects your outcome.



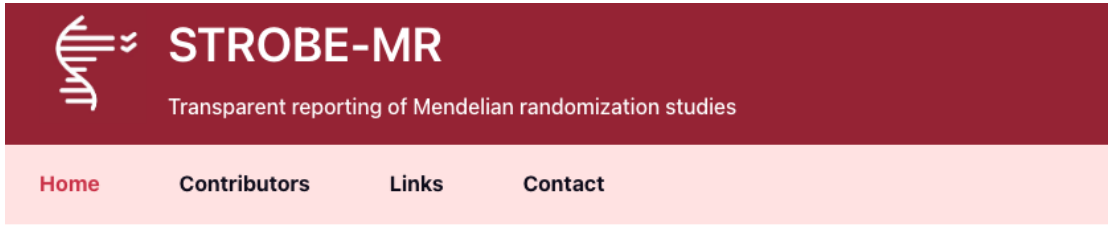
MR methods for handling horizontal pleiotropy

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

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.

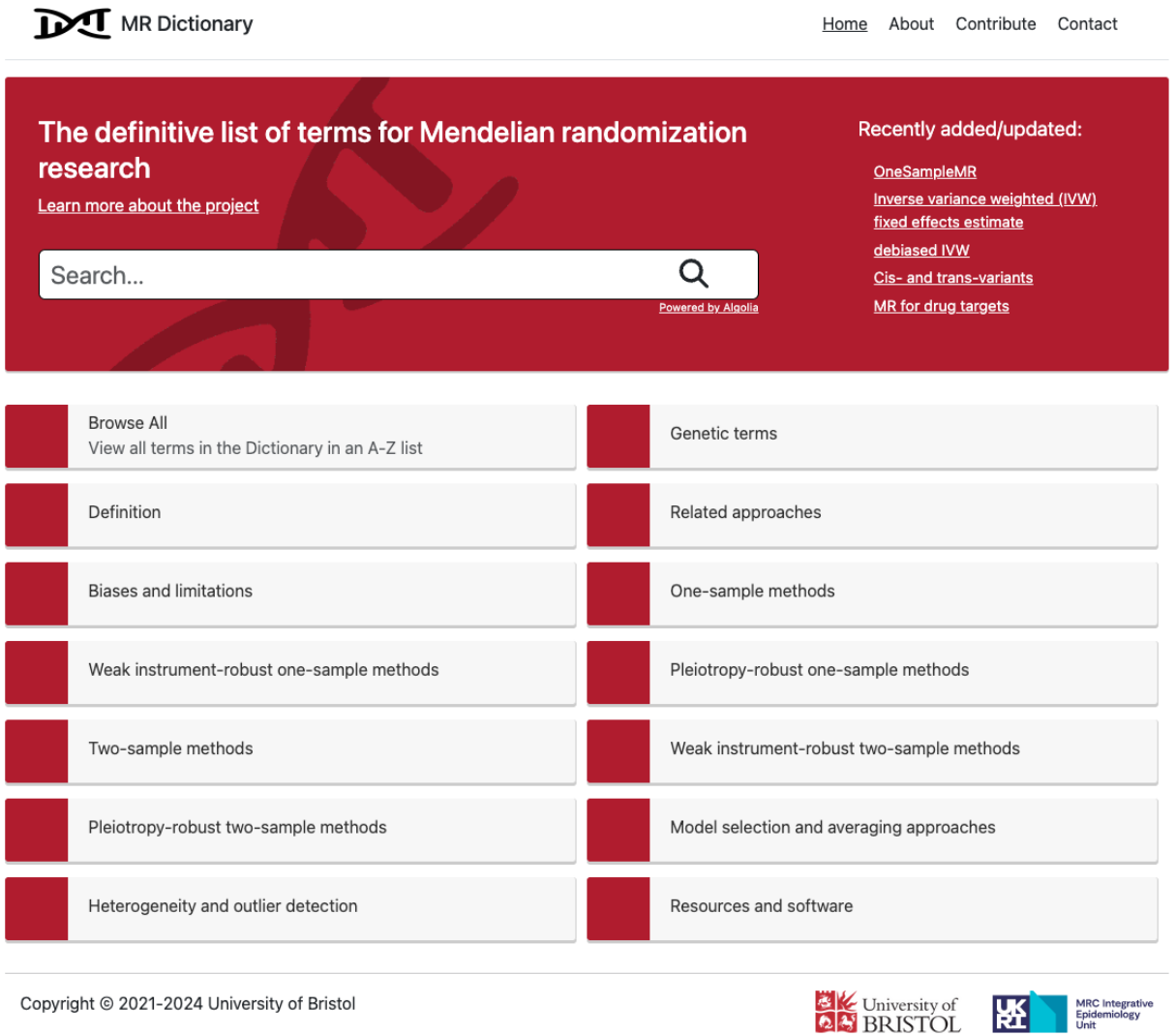
The STROBE-MR **checklist** contains 20 items recommended to address in reports of Mendelian randomization studies.

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



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

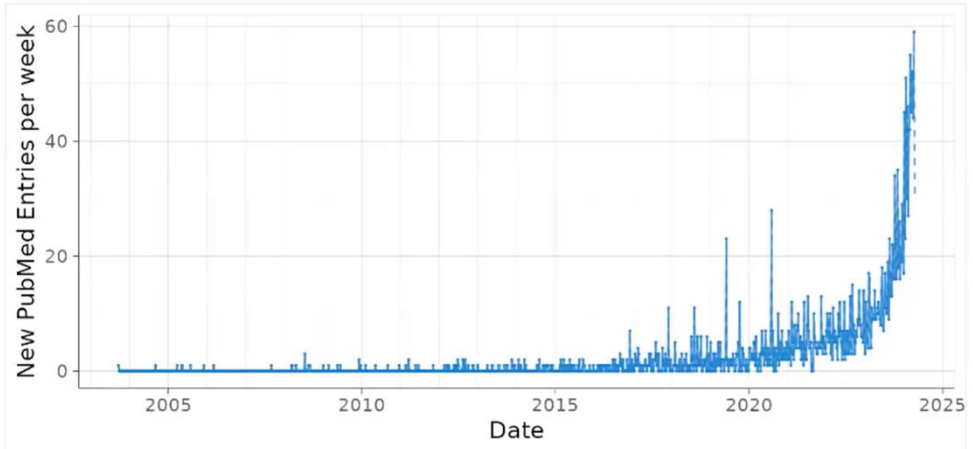
R-CMD-check passing

lifecycle experimental

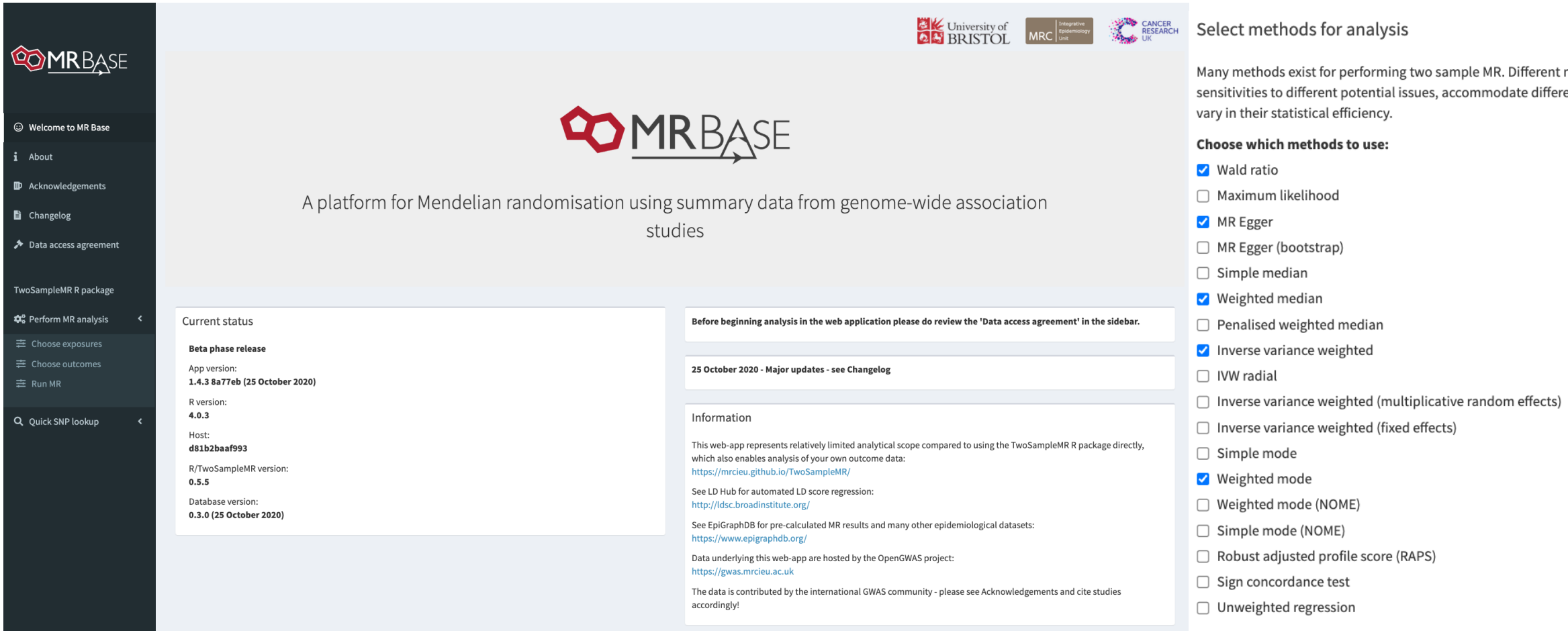
DOI 10.5281/zenodo.10684540

codecov 36%

r-universe 0.6.4



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



Useful References

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