Mendelian Randomization (MR)

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Studies in Genetic Epidemiology

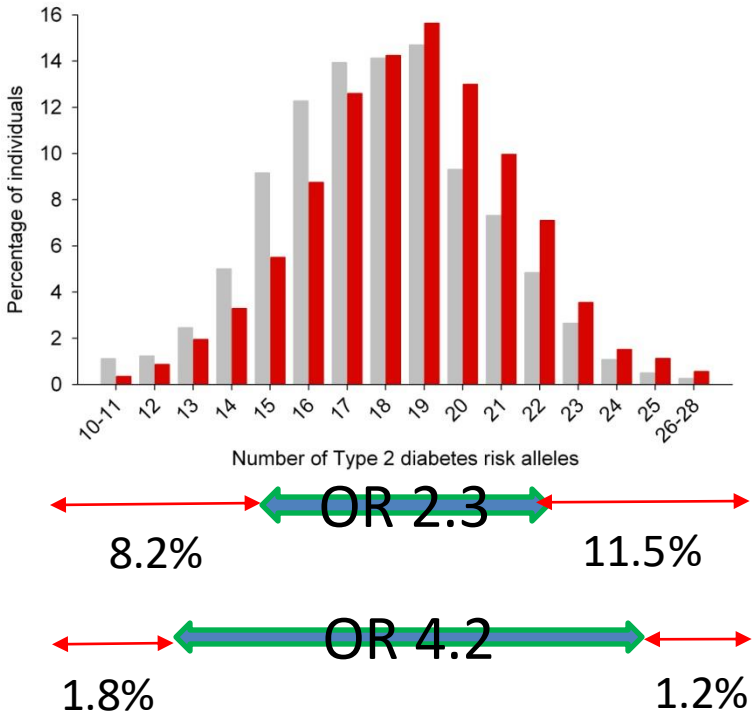
- Linkage analysis using families takes unbiased look at whole genome, but is underpowered for the size of genetic effects we expect to see for many complex genetic traits.

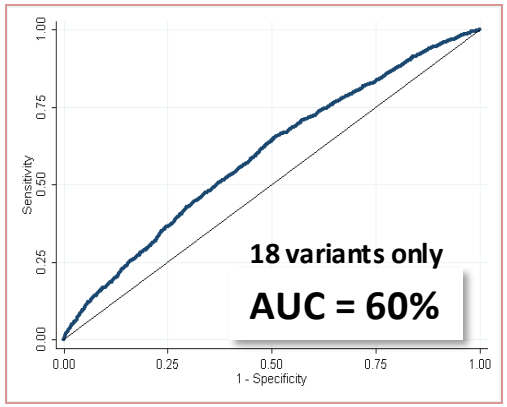
- Candidate-gene association studies have greater power to identify smaller genetic effects, but rely on *a priori* knowledge about disease etiology.

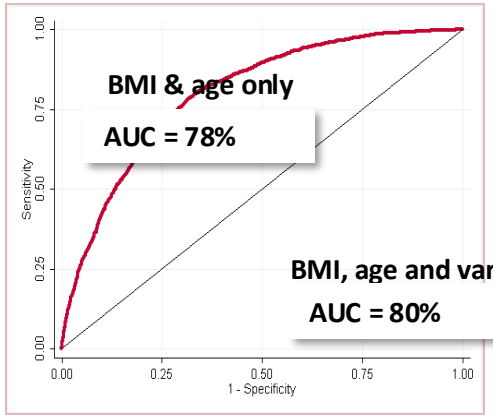
- Genome-wide association studies combine the genomic coverage of linkage analysis with the power of association studies to have much better chance of finding complex trait susceptibility variants.

    - Other advantages: agnostic search, large sample sizes, improved quality of genotyping, rigorous p-value thresholds, replication

Prediction hasn't achieved (yet) full potential







Even with 40 genetic variants prediction is poor

Individual effects are modest

Only ~5-10% of genetic predisposition found

In latest GWAS, 243 genetic variants, explained 18% of T2D risk

Weedon et al, PLOS, 2007
Lango et al, Diabetes 2008
Mahajan et al, Nat Gen 2018

Missing Heritability?

The case of the missing heritability

When scientists opened up the human genome, they expected to find the genetic components of common traits and diseases. But they were nowhere to be seen. **Brendan Maher** shines a light on six places where the missing loot could be stashed away.

Reasons for missing heritability

- "Common disease, common variant" is incorrect – study rarer variants
- Calculation of heritability effects is wrong?
- Not enough common variants of small effect detected
- Structural or other genomic variants more important
- Difficult to analyse gene-gene/gene-environment interactions and in general high-dimensional and systems biology data (i.e., combination of genomic, transcriptomic, proteomic, metabolomic data)

Reasoi

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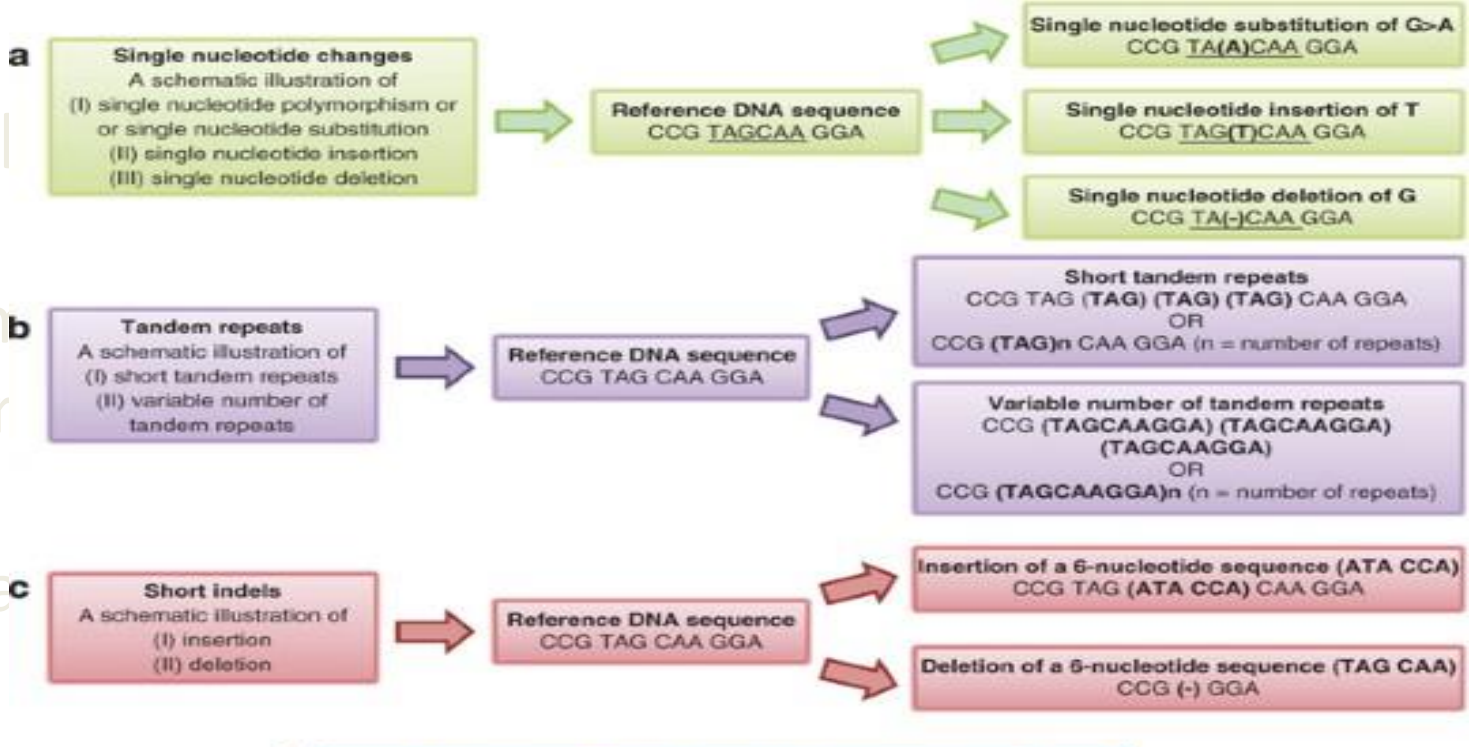
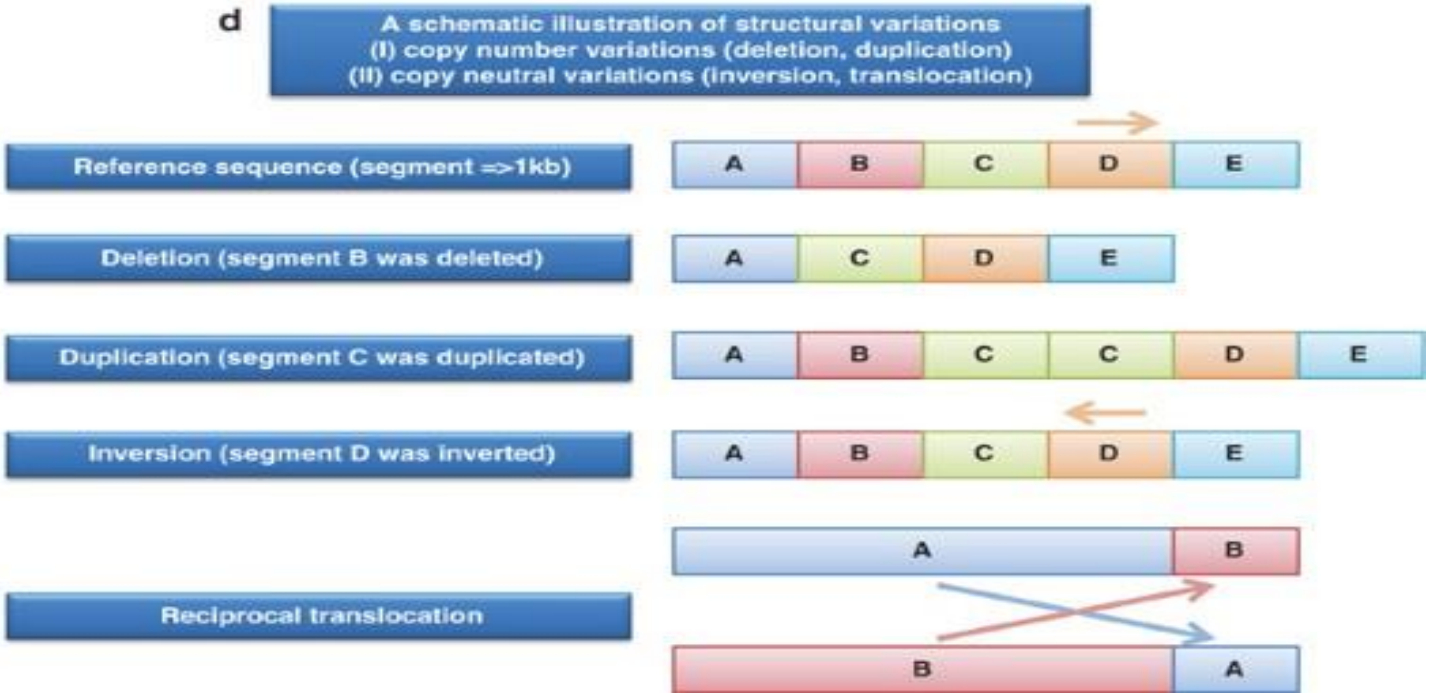




Ways forward…

- Further genetic discovery (denser genotyping)
- Better characterization of validated genes
- Gene-Environment & Gene-Gene interaction
- Use genes for causal inference (Mendelian randomization)
- Whole genome sequencing
- Systems biology approaches
- Development of clinically useful risk prediction models
- Other translation

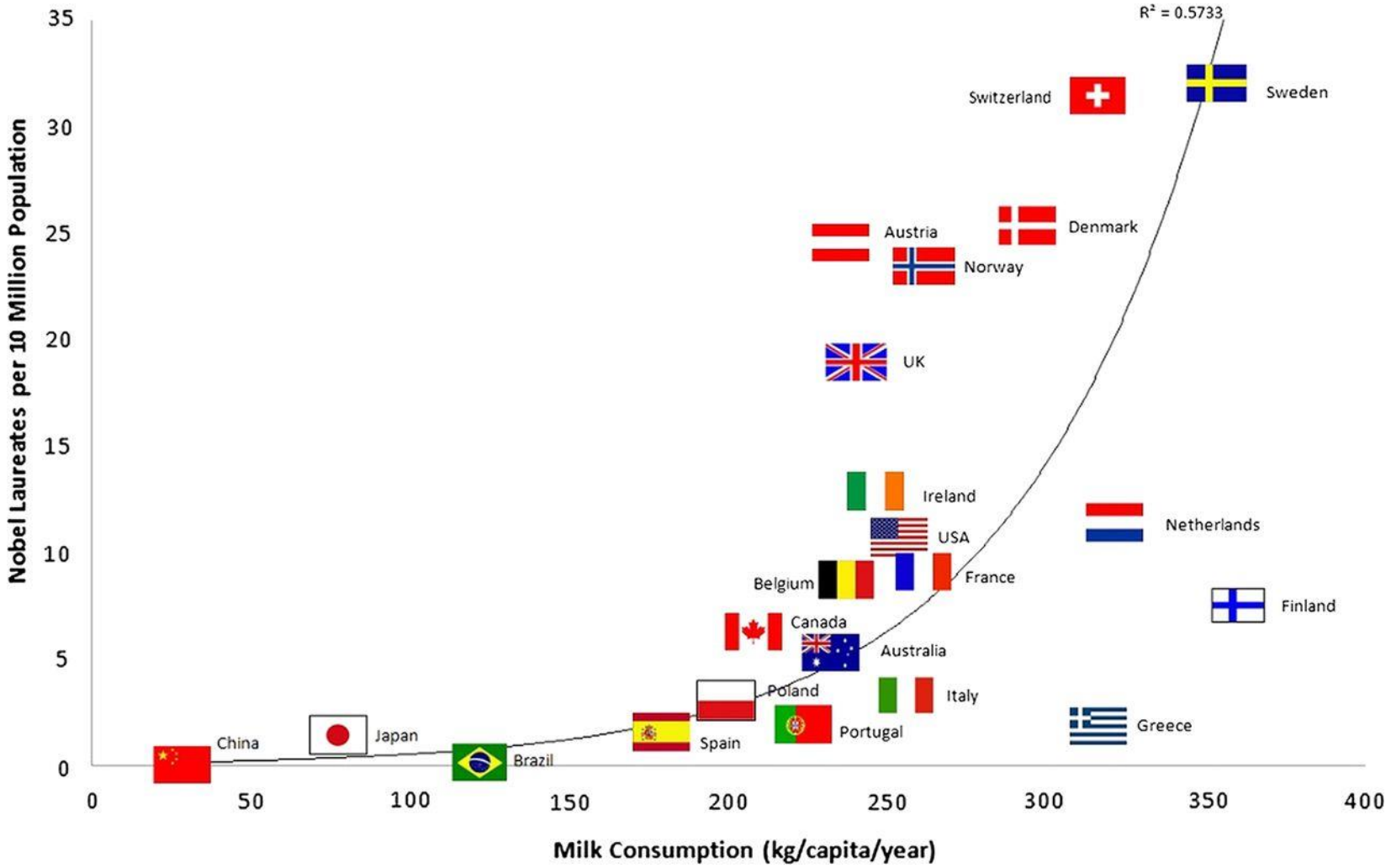
Learning Objectives

- What is the concept and aim of MR?
- Describe the assumptions of this methodology
- How are MR effects estimated?
- Appreciate the strengths, promises and limitations of MR.

Outline

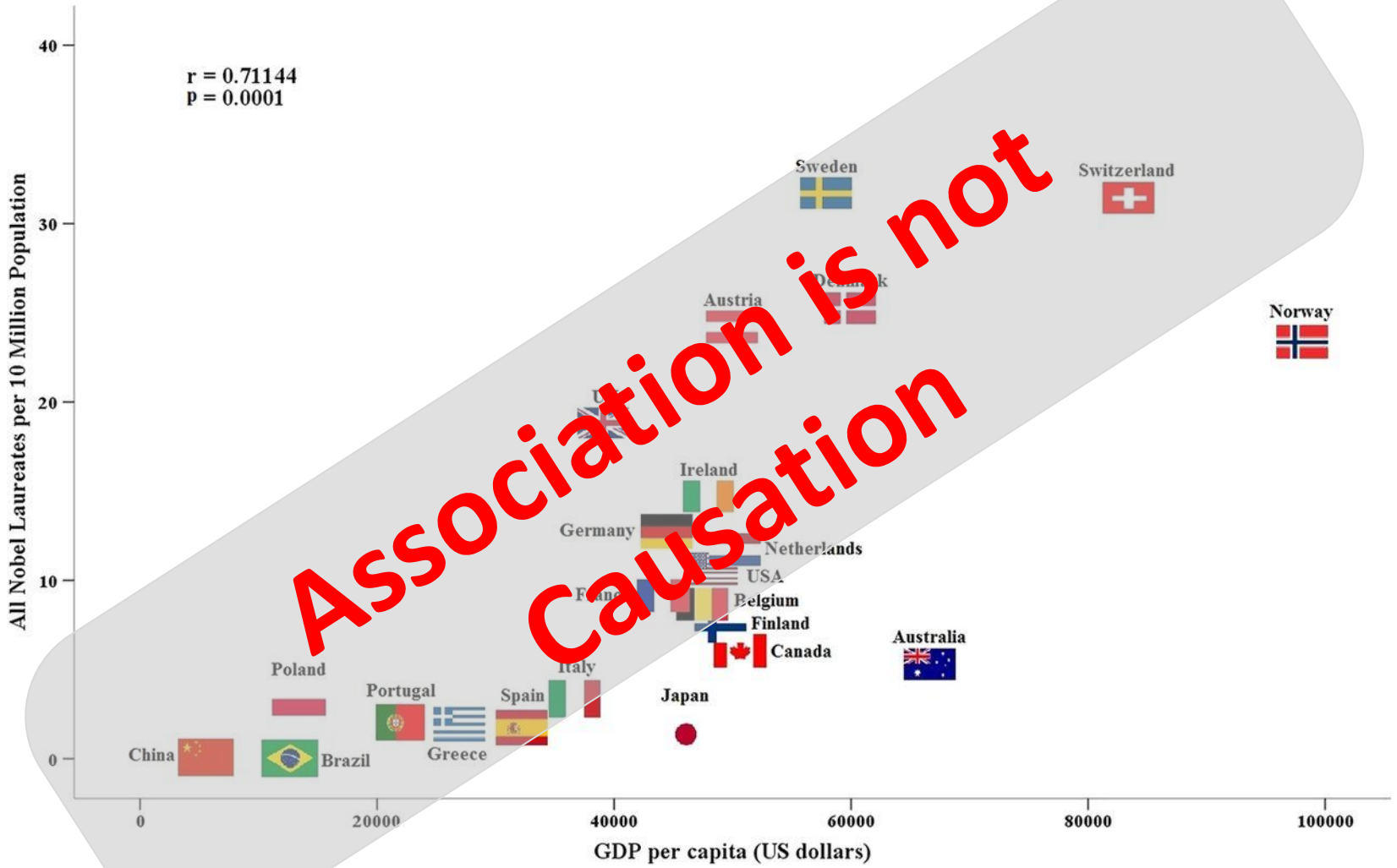
- Mendelian Randomization
  - Conceptual Overview
  - Assumptions
  - Design of MR studies
  - Effect estimation
  - Methods to assess pleiotropy
  - Examples
  - Limitations and Current Advances

Correlation between countries' annual per capita milk consumption and the number of Nobel laureates per 10 million population.



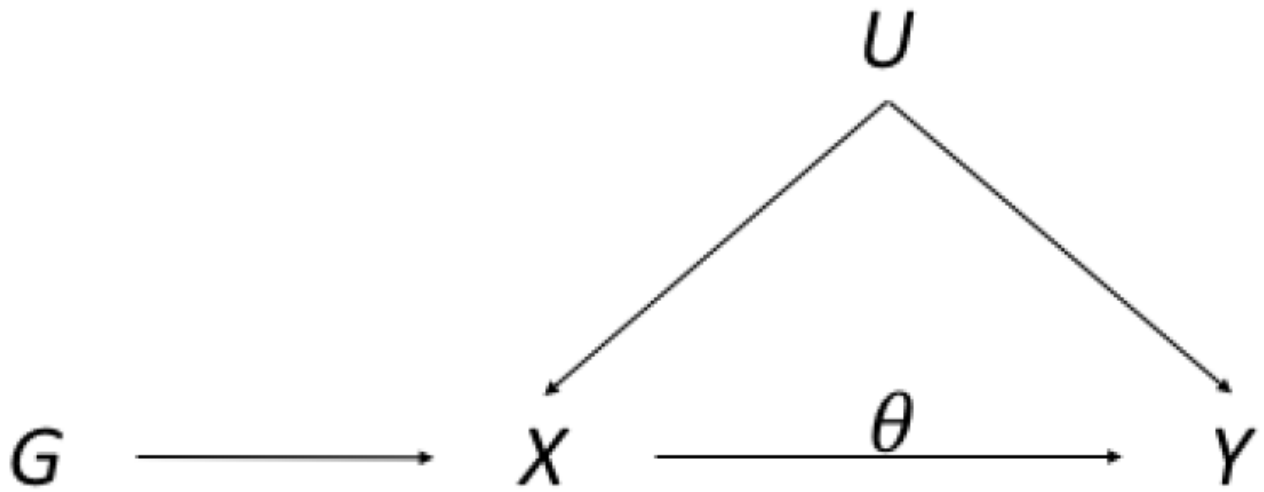
Linthwaite S , and Fuller G N Pract Neurol 2013;13:63

Correlation between countries' gross domestic product per capita and the number of all Nobel laureates per 10 million population (23 countries).



Li J Pract Neurol 2014;14:e1

Graphic representation of Mendelian randomization



- G: Genetic variant as **instrumental variable (IV)**
- X: Risk factor
- Y: Outcome
- θ: Causal effect of X on Y
- U: Confounder (unobserved)

Definition of Mendelian randomization (MR)

- *"The use of genetic data on human participants in an observational setting to evaluate the potential causal nature of a modifiable risk factor"*
  - Recent examples of causal effects
    - Blood pressure, obesity, LDL-C, IL-6 and CVD
  - Recent examples of lack of causal effects
    - CRP, HDL-C and CVD

Burgess S, et al. J Compar Effect Res 2013;2:209-212

Attributes of Mendelian randomization

- Certain genetic polymorphisms produce phenotypes which mimic (reflect, serve as proxies for) the effect of environmental exposures

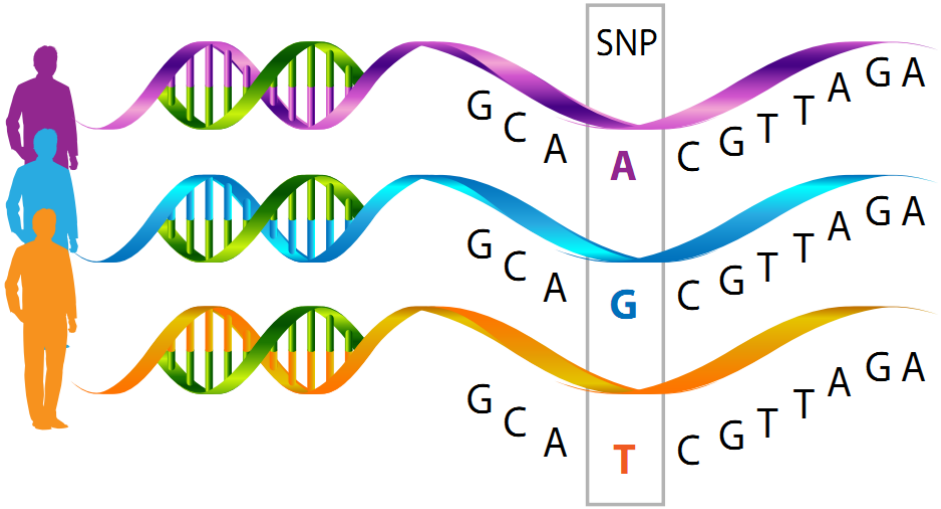
- Allelic variants mimicking environmental exposures (genetic instruments, instrumental variables (IV))
  - *IL6* gene for serum IL-6
  - Vitamin D metabolizing genes for serum 25(OH)D
  - *ALDH2* gene for alcohol intake
  - Lactase persistence gene for dairy product intake
  - *HFE* mutations for high serum iron

- Because of random assortment of alleles, MR reduces bias due to confounding
- MR also largely avoids reverse causation bias

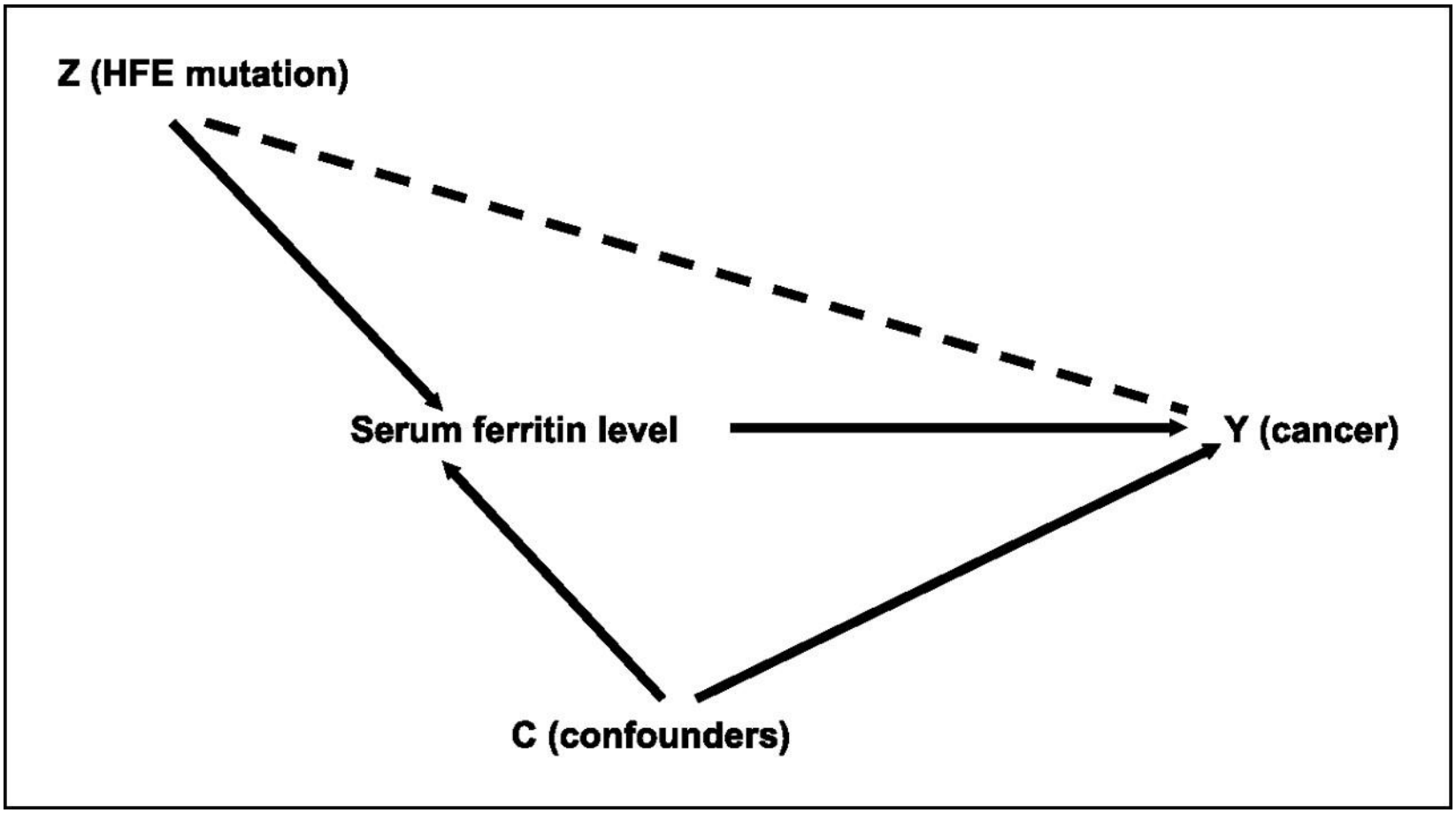
Mendel's law

- "Mendelian randomization" refers to the random assortment of genes transferred from parent to offspring at the time of gamete formation.

- "Mendelian randomization approach" is an application of the instrumental variable approach for causal inference.



A directed acyclic graph depicting how the *HFE* gene can be used as a proxy (instrumental variable) for serum ferritin in a Mendelian randomization study of cancer.



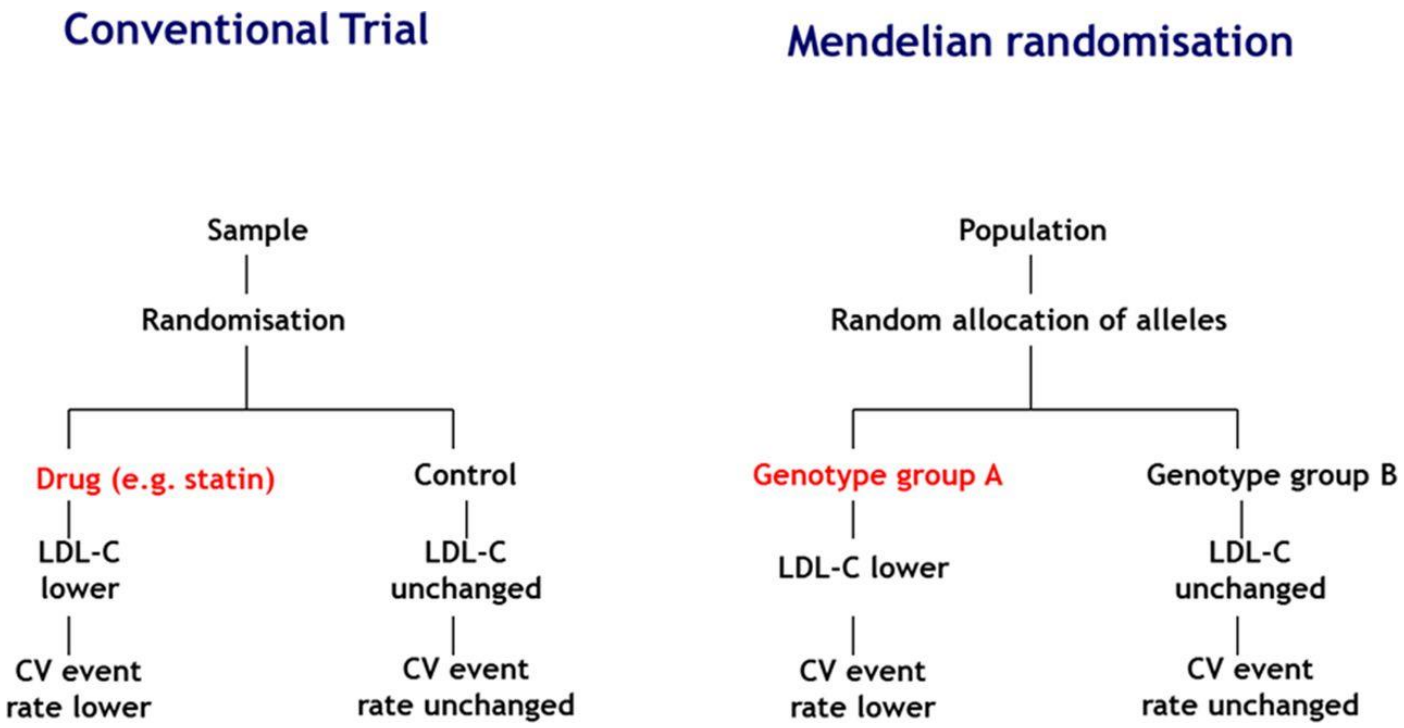
Schatzkin A et al. Cancer Prev Res 2009;2:104-113

Example of instrumental variables (IVs)

- Genetic profile (Mendelian randomization)
- Random allocation in trials
- Regulation changes (tax)
- Physician preference
- Lottery to serve in the army

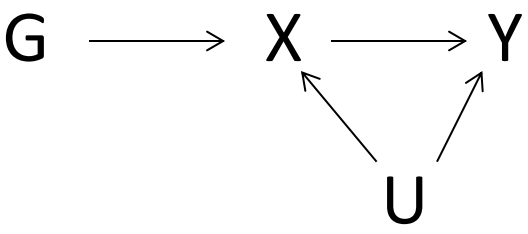
MR is analogous to a RCT

- MR is a natural experiment in observational data

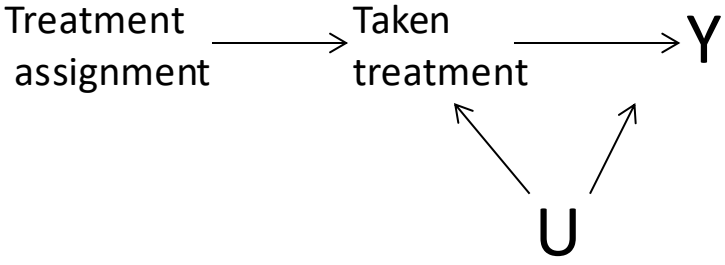


Assumptions of MR

1. The genetic marker is associated with the exposure (relevance).
2. The genetic marker is independent of factors that confound the exposure-outcome association (exchangeability).
3. The genetic marker is independent of the outcome given the exposure and all confounders (exclusion restriction).



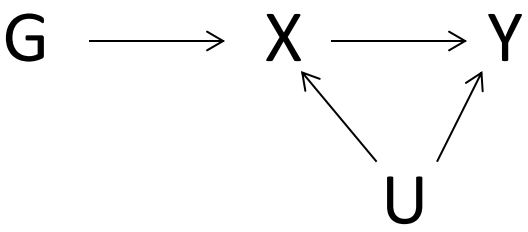
Analogy to an RCT



VanderWeele TJ, et al. Epidemiology 2014;25:427-435

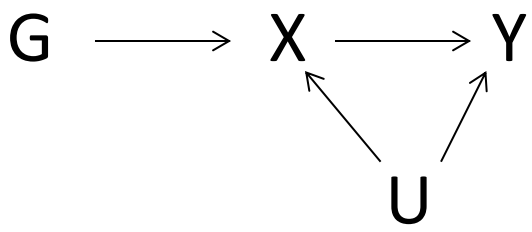
Assumption 1: Relevance

- Genetic variants should be strongly associated with the exposure
- How to select genetic variants as instrumental variables:
  - F-statistic>10
  - Genome-wide significance: p-value<5×10^{-8}
- Weak instruments can induce bias



Weak instrument bias

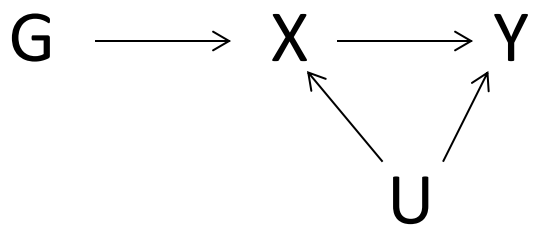
- The IV strength is measured by the F statistic from the X on G regression.
- If F<10, then weak instrument.



$$F = \frac{R^2(n-1-k)}{(1-R^2)k}$$

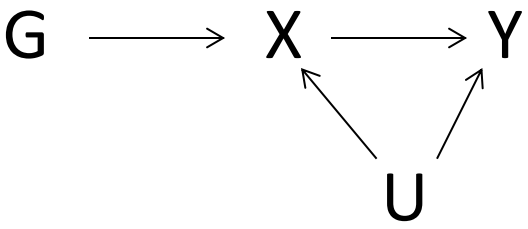
Assumption 2: Exchangeability

- Because genotypes are assigned randomly from parents to offspring at meiosis, the genotype distribution should be independent of environmental confounders.
- Assumption: random mating
- Population stratification (genetic confounding) can violate this assumption



Assumption 3: Exclusion restriction

- There is no other arrow from G to Y than via X
- Reasons for violation:
  - Pleiotropy (horizontal)
  - G*E interaction
  - G*G interaction
  - Linkage disequilibrium
  - Population stratification
  - Other



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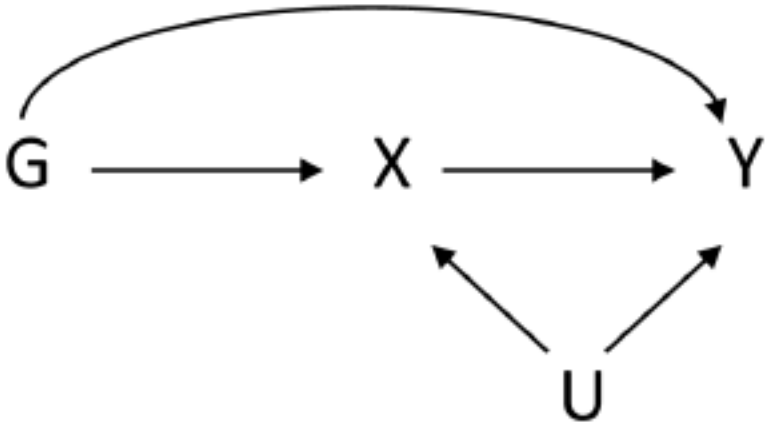
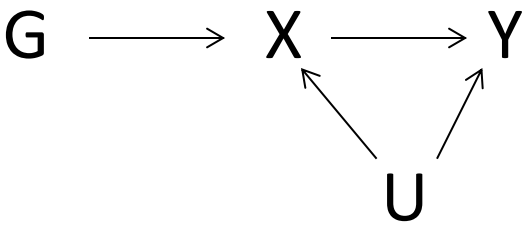
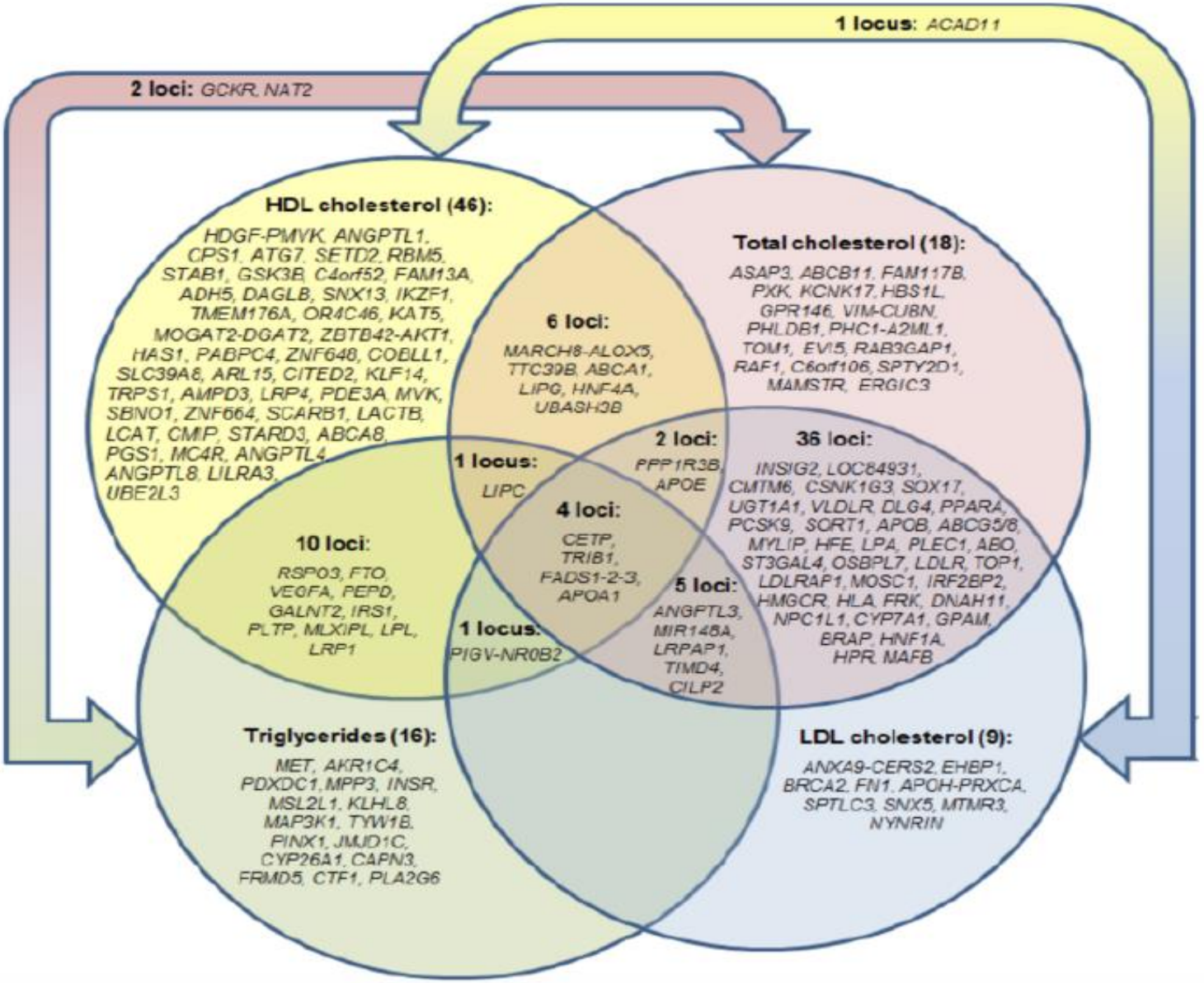


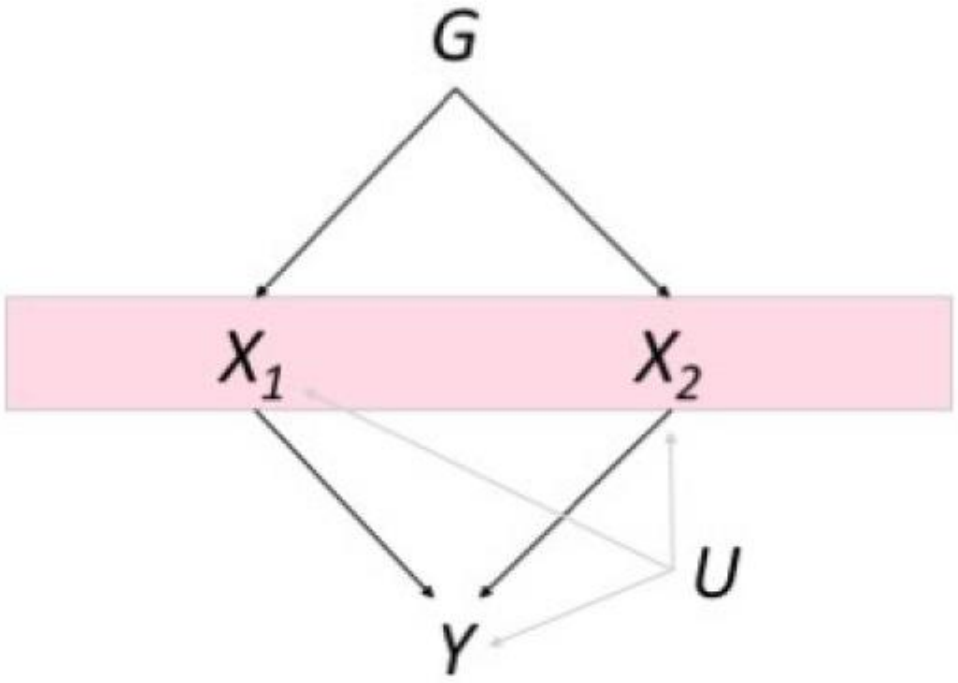


Example of pleiotropy: blood lipids

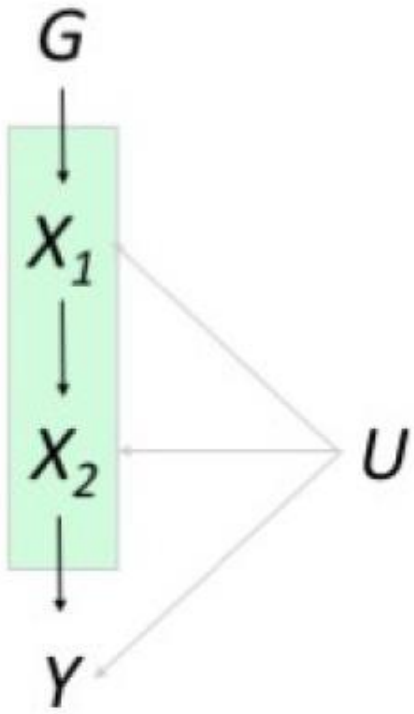


Horizontal vs. vertical pleiotropy

Horizontal pleiotropy:
→ Different pathways



Vertical pleiotropy:
→ The same pathway



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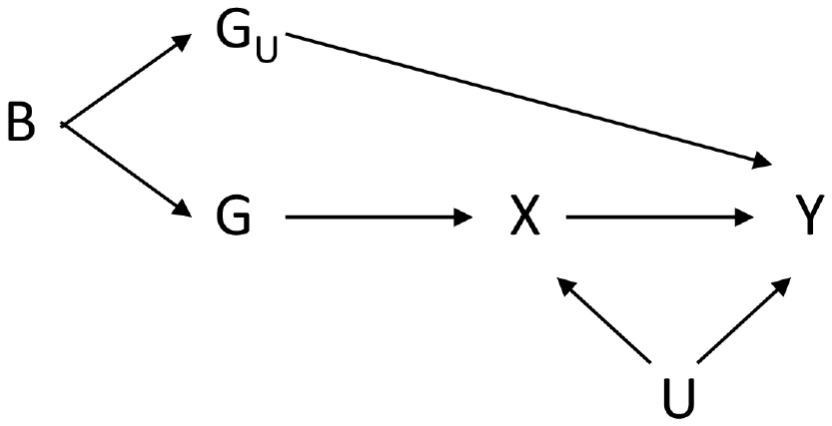
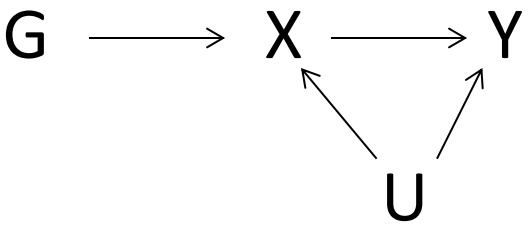


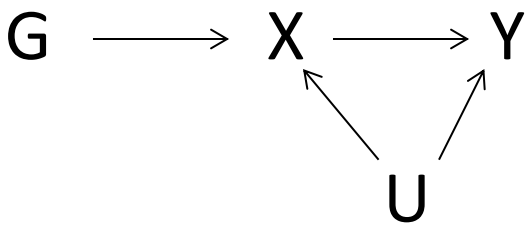


Evaluating the MR assumptions:
no way to prove that IV2-3 definitively hold

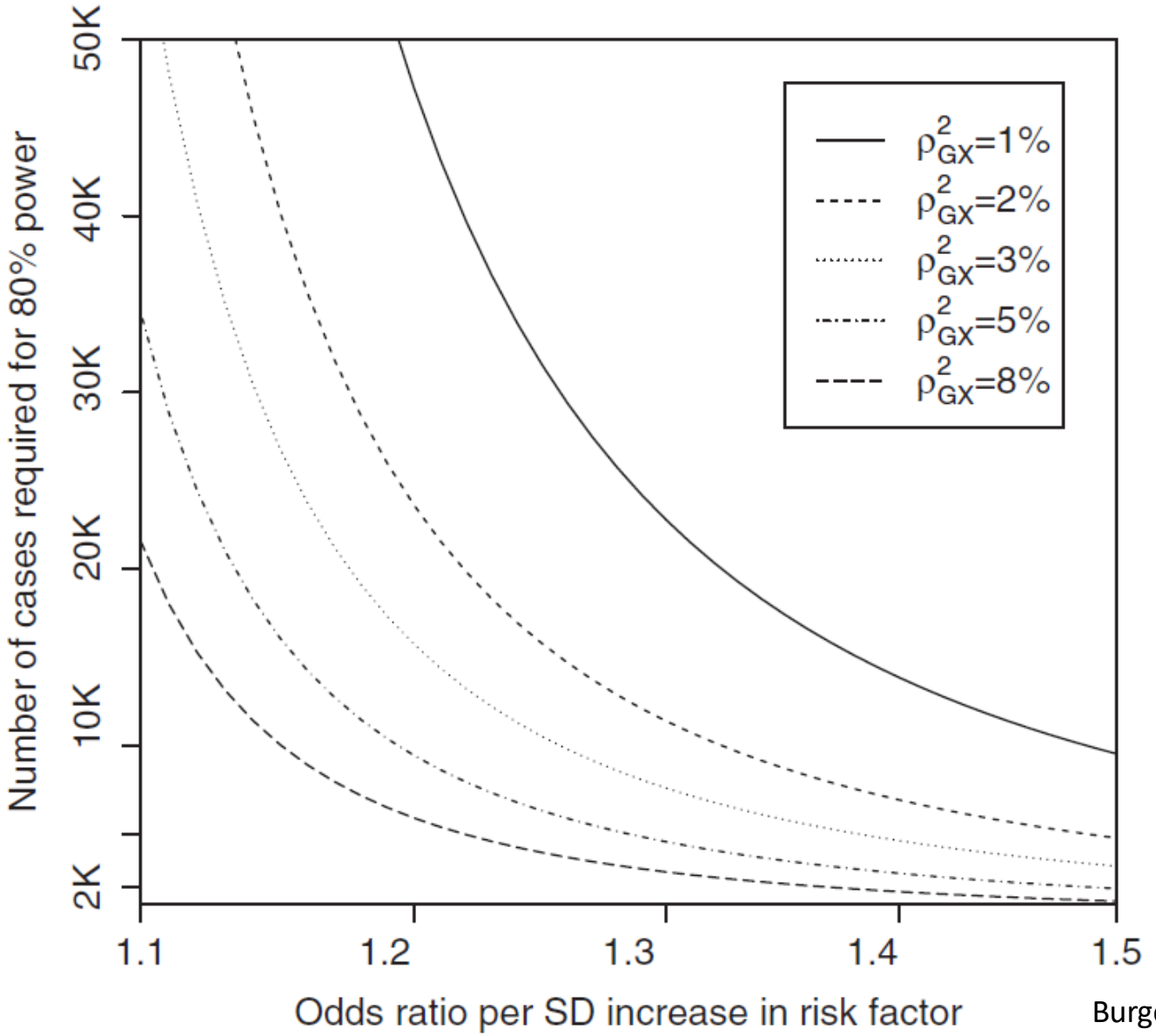
1. The genetic marker is associated with the exposure.
   - It can be easily evaluated in a dataset

2. The genetic marker is independent of factors that confound the exposure-outcome association.
   - Test whether G is associated with measured U factors

3. The genetic marker is independent of the outcome given the exposure and all confounders.
   - Adjust for X in the G-Y association, but beware of collider bias (need to adjust also for U)



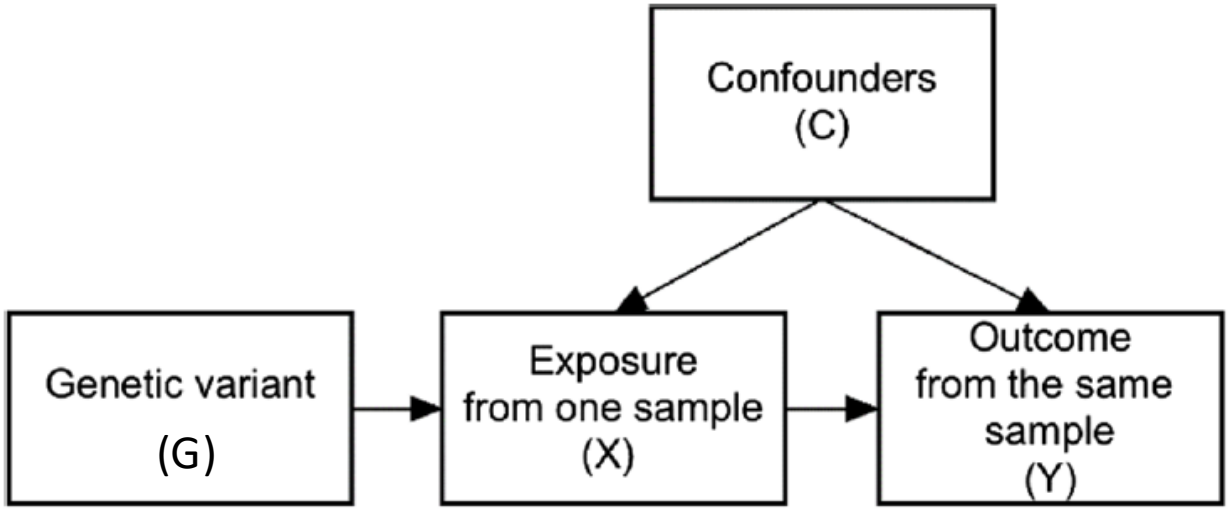
Power and sample size in MR studies



Burgess S. Int J Epidemiol 2014:922-929

Designs of MR studies

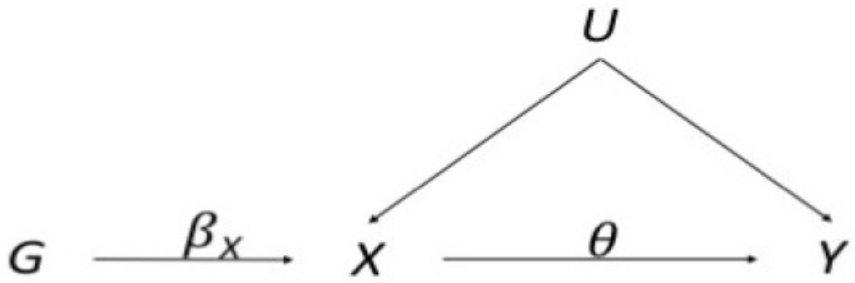
- In the traditional MR setting (one-sample MR), data on G, X and Y are available for all participants.
- Sub-sample MR methods
- Two-sample MR methods



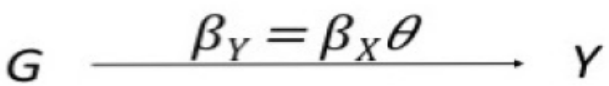
Zheng J, et al. Curr Epidemiol Rep 2017;4:330-345

Instrumental variable (IV) estimators in one-sample MR

- "Wald" or "ratio" method (can accommodate one IV)
  - β_X: The association of G with the risk factor X
  - β_Y: The association of G with the outcome Y



If the exclusion restriction assumptions holds, the effect of G on Y can be decomposed into



Palmer TM, et al. Am J Epidemiol 2011;173:1392-1403

Instrumental variable (IV) estimators in one-sample MR

- "Wald" or "ratio" method (can accommodate one IV)

The ratio estimate $\hat{\theta}_{ratio}$

$$\hat{\theta}_{ratio} = \frac{\beta_Y}{\beta_X}$$

Instrumental variable (IV) estimators in one-sample MR

- Two-stage least squares method (can accommodate multiple IVs)

1. Stage: Predict the risk factor X based on the genotype G
   - Linear regression of G on X

$$X = \beta_X G + \epsilon \tag{1}$$

   - Predicted values $\hat{X}$ based on genetic model

$$\hat{X} = \beta_X G \tag{2}$$

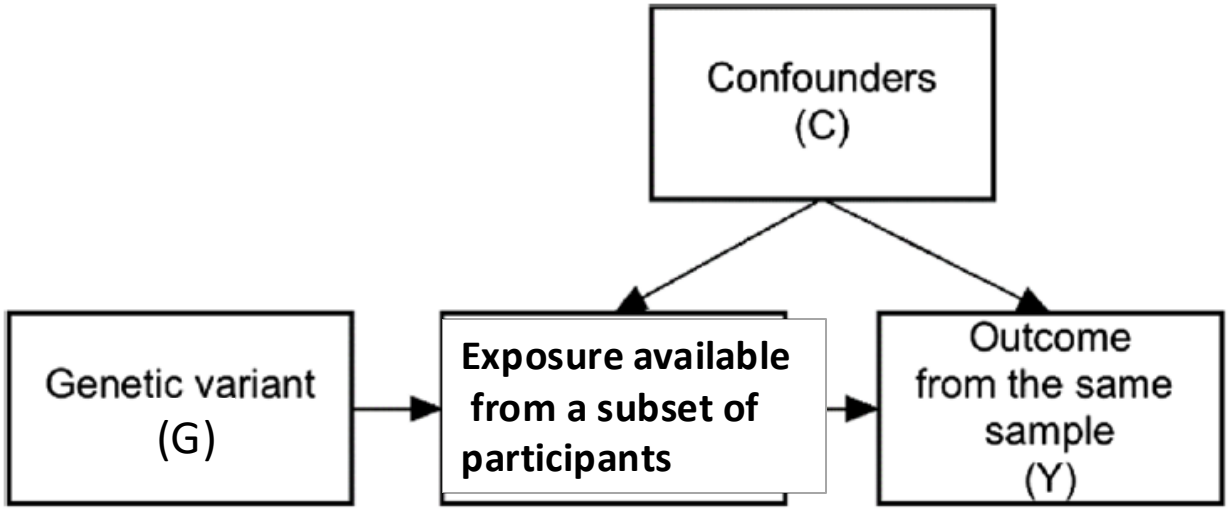
2. Stage: Linear regression of $\hat{X}$ on Y

$$Y = \hat{\theta}_{2SLS}\hat{X} + \epsilon \tag{3}$$

$\hat{\theta}_{2SLS}$ is the two-stage least squares causal effect estimate for X on Y.

Designs of MR studies

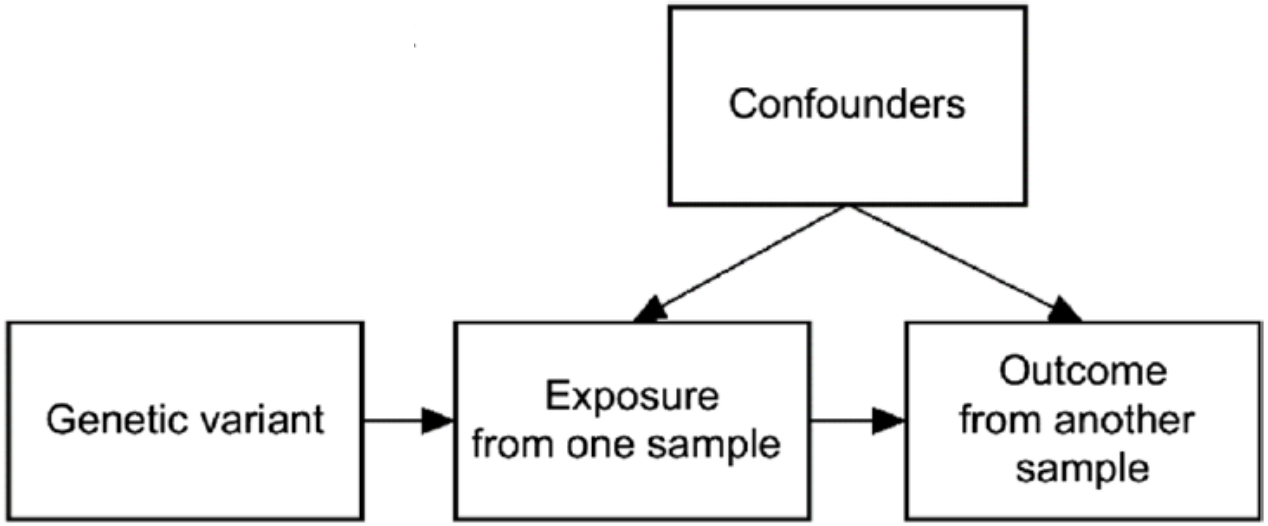
- In the traditional MR setting (one-sample MR), data on G, X and Y are available for all participants.
- Sub-sample MR methods
- Two-sample MR methods



Zheng J, et al. Curr Epidemiol Rep 2017;4:330-345

Designs of MR studies

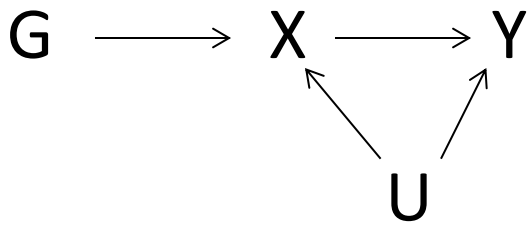
- In the traditional MR setting (one-sample MR), data on G, X and Y are available for all participants.
- Sub-sample MR methods
- Two-sample MR methods (could use summary literature data)



Zheng J, et al. Curr Epidemiol Rep 2017;4:330-345

Sub-sample and 2-sample MR methods

- Exposure data available for a subset (or an independent set) of participants
- >90% of the maximum power can be achieved by obtaining exposure data on only 20% of the sample
- Power for MR is most efficiently increased by increasing the sample size of the outcome-gene association



Pierce BL, et al. Am J Epidemiol 2013;178:1177-1184

Instrumental variable (IV) estimators in two-sample MR

- "Wald" or "ratio" method (can accommodate one IV)
- Assumption: homogeneous effects in the two cohorts

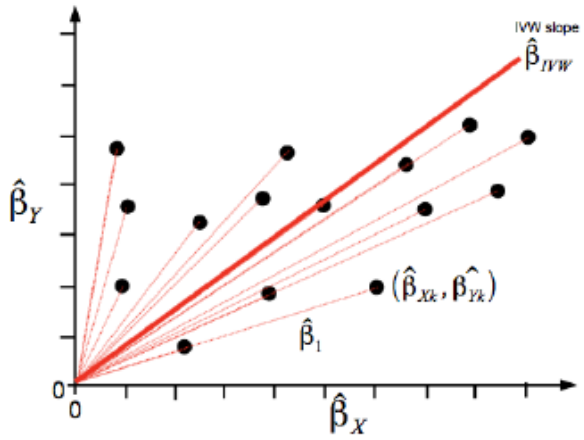
The ratio estimate $\hat{\theta}_{ratio}$

$$\hat{\theta}_{ratio} = \frac{\beta_Y}{\beta_X}$$

Instrumental variable (IV) estimators in two-sample MR

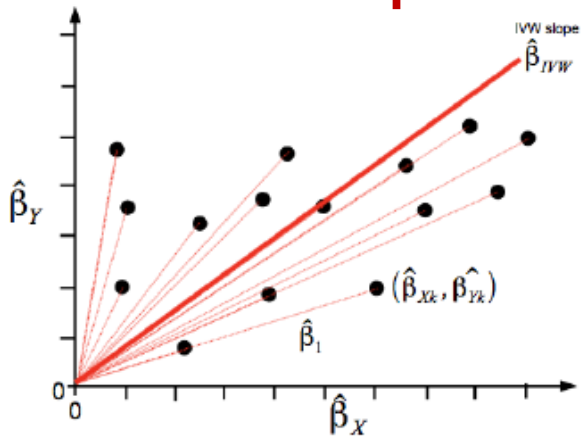
- Inverse variance weighting of individual IV effects (can accommodate multiple IVs)

$$\hat{\theta}_{IVW} = \frac{\sum_{j=1}^{n} \hat{\theta}_j / var(\hat{\theta}_j)}{\sum_{j=1}^{n} 1 / var(\hat{\theta}_j)}$$



- Assumption: The genetic variants are independent and thus the ratio estimates are independent

Instrumental variable (IV) estimators in two-sample MR

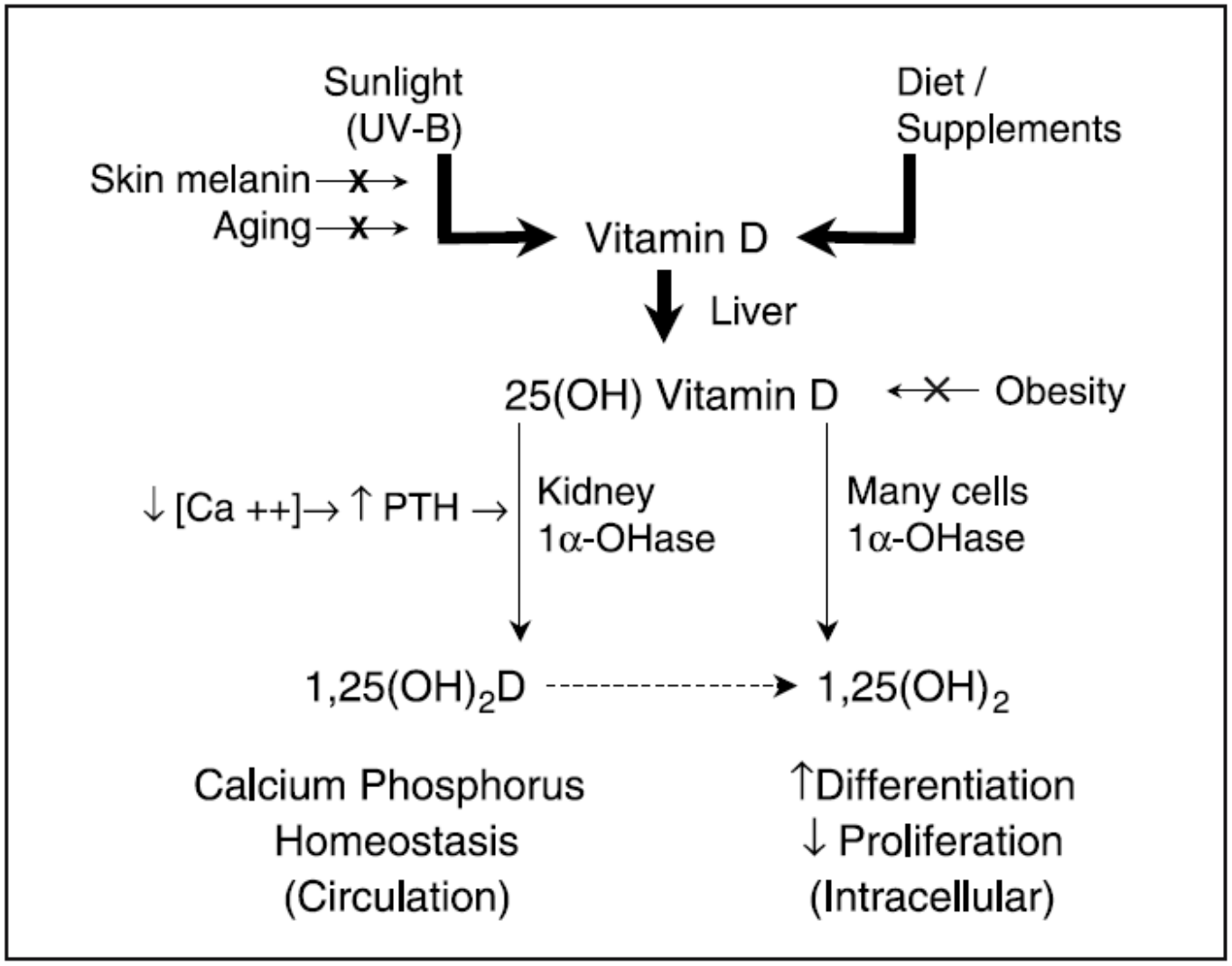


- The IVW estimate θ_{IVW} is a weighted average of slope estimates $\theta 1$, ..., θn
- Equivalent to fitting a weighted regression model
- The regression coefficient θreg is an estimate of the causal effect θ: $\beta_{Y_j} = \hat{\theta}_{reg}\beta_{X_j} + \epsilon_j$
- Note that there is no intercept which is a consequence of the IV assumptions (e.g. no pleiotropy)

Example: Circulating vitamin D concentrations (25(OH)D) and cancer risk

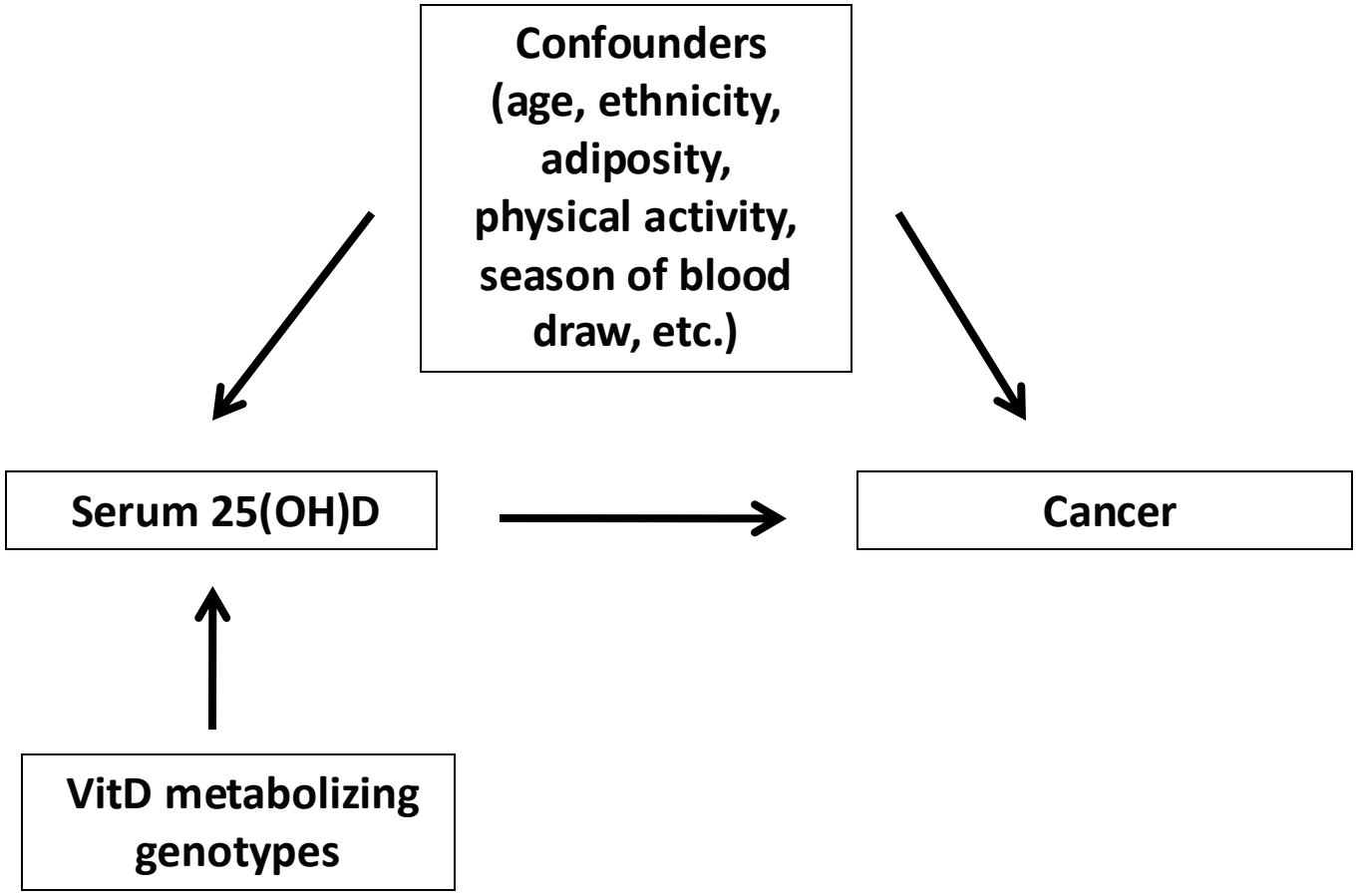
- Ample biological evidence for an anti-cancer role of 25(OH)D
  - Vitamin D metabolites control cellular growth and differentiation.
  - Administration of vitamin D analogues inhibits progression of several cancers in animal models and cell lines.
- Epidemiological studies have been inconclusive.
- Vitamin D supplementation trials currently provide no firm evidence for increase or decrease of cancer occurrence.

Proposed mechanism

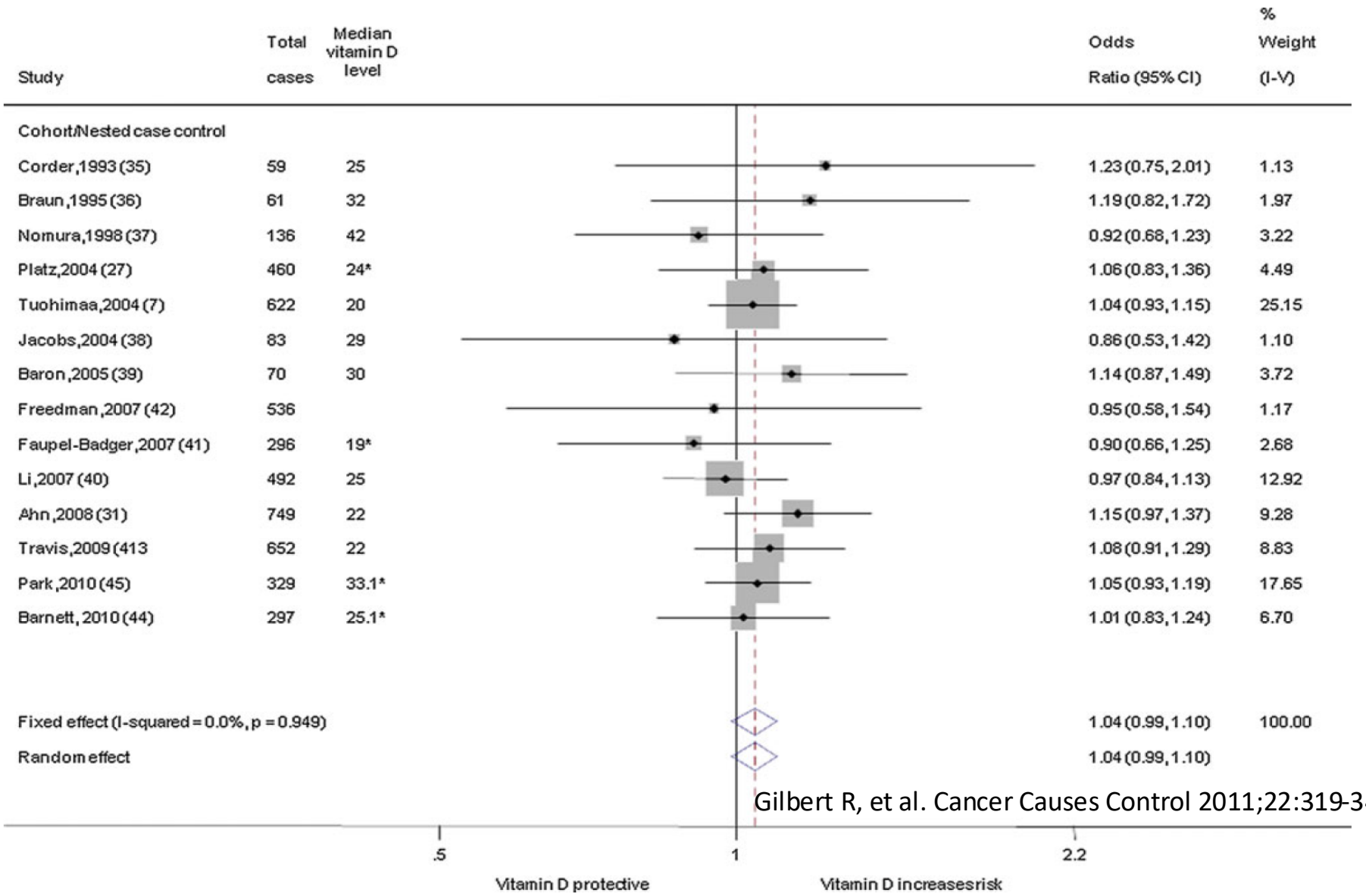


Giovannucci E. Cancer Causes Control 2005;16:83-95

Causal diagram: Vitamin D and cancer

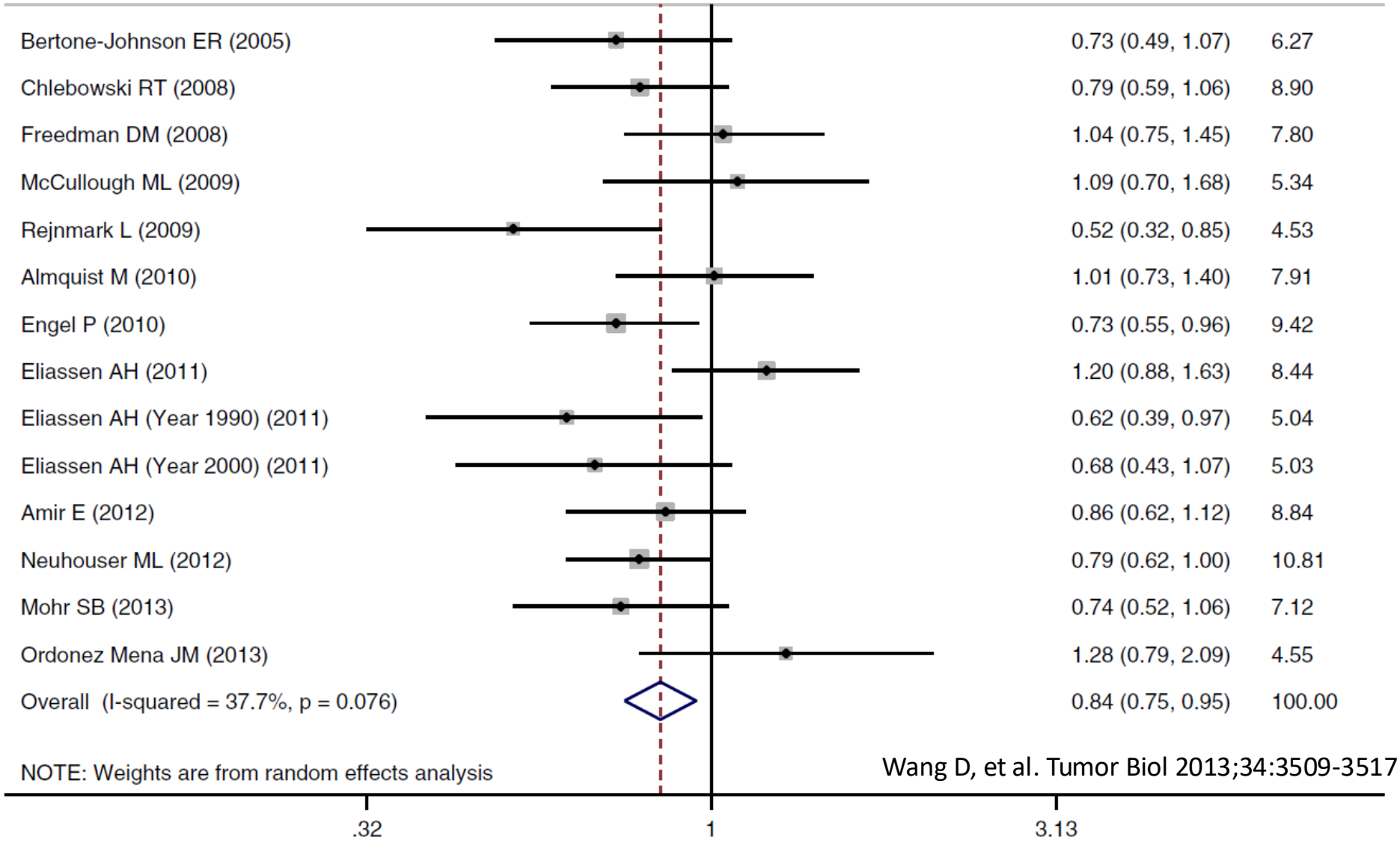


25(OH)D and prostate cancer risk



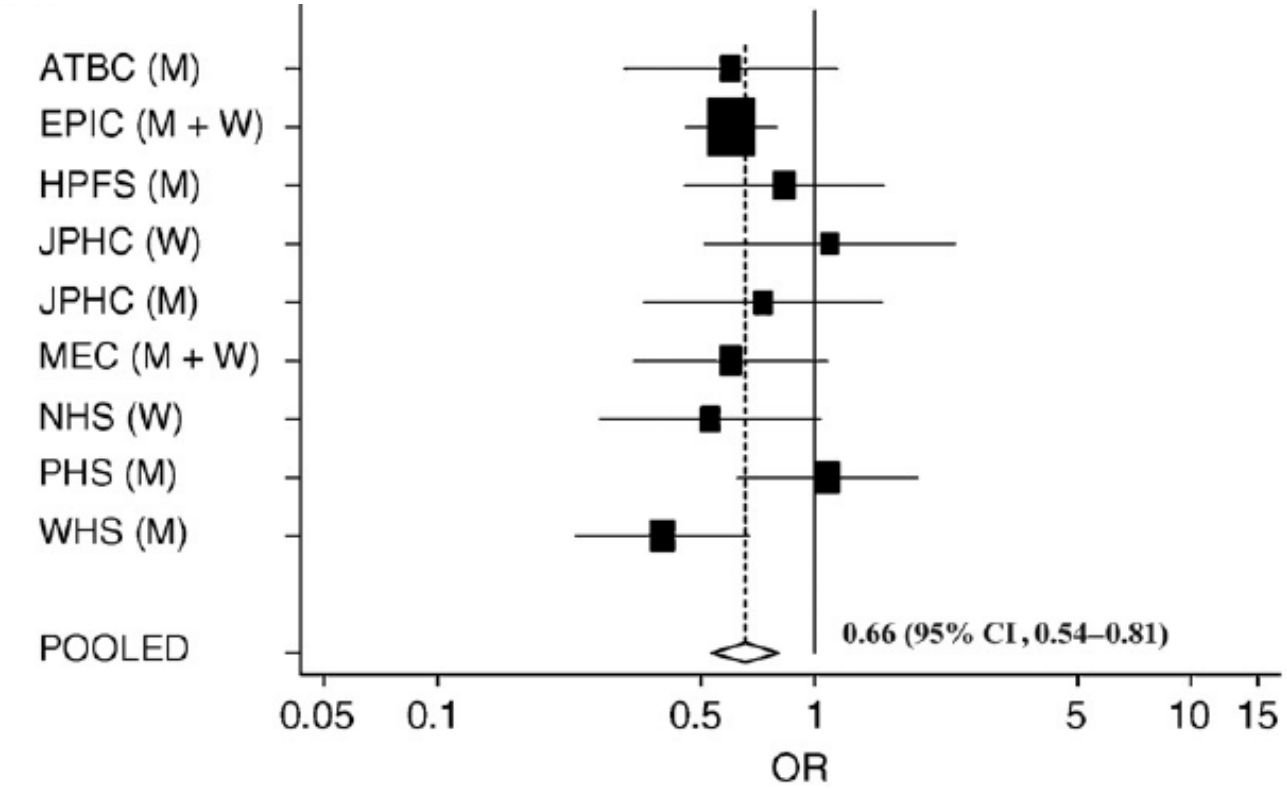
Gilbert R, et al. Cancer Causes Control 2011;22:319-340

25(OH)D and breast cancer risk



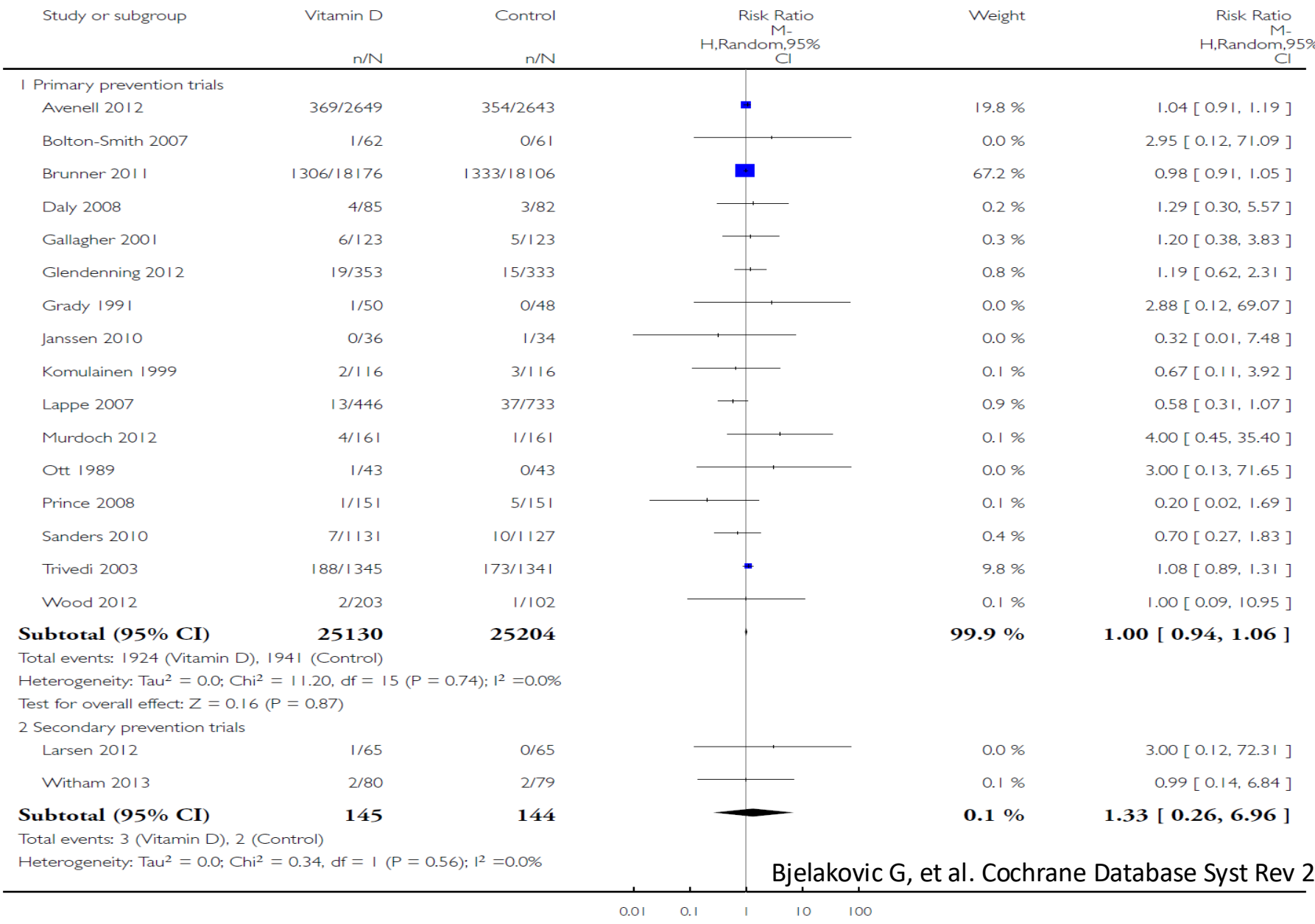
Wang D, et al. Tumor Biol 2013;34:3509-3517

25(OH)D and colorectal cancer risk



Lee JE, et al. Cancer Prev Res 2011;4:735-743

Vitamin D supplements and cancer risk



Study or subgroup	Vitamin D n/N	Control n/N	Risk Ratio M-H,Random,95% CI	Weight	Risk Ratio M-H,Random,95% CI
1 Primary prevention trials					
Avenell 2012	369/2649	354/2643		19.8 %	1.04 [0.91, 1.19]
Bolton-Smith 2007	1/62	0/61		0.0 %	2.95 [0.12, 71.09]
Brunner 2011	1306/18176	1333/18106		67.2 %	0.98 [0.91, 1.05]
Daly 2008	4/85	3/82		0.2 %	1.29 [0.30, 5.57]
Gallagher 2001	6/123	5/123		0.3 %	1.20 [0.38, 3.83]
Glendenning 2012	19/353	15/333		0.8 %	1.19 [0.62, 2.31]
Grady 1991	1/50	0/48		0.0 %	2.88 [0.12, 69.07]
Janssen 2010	0/36	1/34		0.0 %	0.32 [0.01, 7.48]
Komulainen 1999	2/116	3/116		0.1 %	0.67 [0.11, 3.92]
Lappe 2007	13/446	37/733		0.9 %	0.58 [0.31, 1.07]
Murdoch 2012	4/161	1/161		0.1 %	4.00 [0.45, 35.40]
Ott 1989	1/43	0/43		0.0 %	3.00 [0.13, 71.65]
Prince 2008	1/151	5/151		0.1 %	0.20 [0.02, 1.69]
Sanders 2010	7/1131	10/1127		0.4 %	0.70 [0.27, 1.83]
Trivedi 2003	188/1345	173/1341		9.8 %	1.08 [0.89, 1.31]
Wood 2012	2/203	1/102		0.1 %	1.00 [0.09, 10.95]
Subtotal (95% CI)	**25130**	**25204**		**99.9 %**	**1.00 [0.94, 1.06]**
Total events: 1924 (Vitamin D), 1941 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 11.20$, df = 15 (P = 0.74); $I^2 = 0.0\%$					
Test for overall effect: Z = 0.16 (P = 0.87)					
2 Secondary prevention trials					
Larsen 2012	1/65	0/65		0.0 %	3.00 [0.12, 72.31]
Witham 2013	2/80	2/79		0.1 %	0.99 [0.14, 6.84]
Subtotal (95% CI)	**145**	**144**		**0.1 %**	**1.33 [0.26, 6.96]**
Total events: 3 (Vitamin D), 2 (Control)					
Heterogeneity: $Tau^2 = 0.0$; $Chi^2 = 0.34$, df = 1 (P = 0.56); $I^2 = 0.0\%$					

Bjelakovic G, et al. Cochrane Database Syst Rev 2014

MR study of 25(OH)D and risk of 7 cancers

Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study

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ABSTRACT

OBJECTIVE

To determine if circulating concentrations of vitamin D are causally associated with risk of cancer.

DESIGN

Mendelian randomisation study.

SETTING

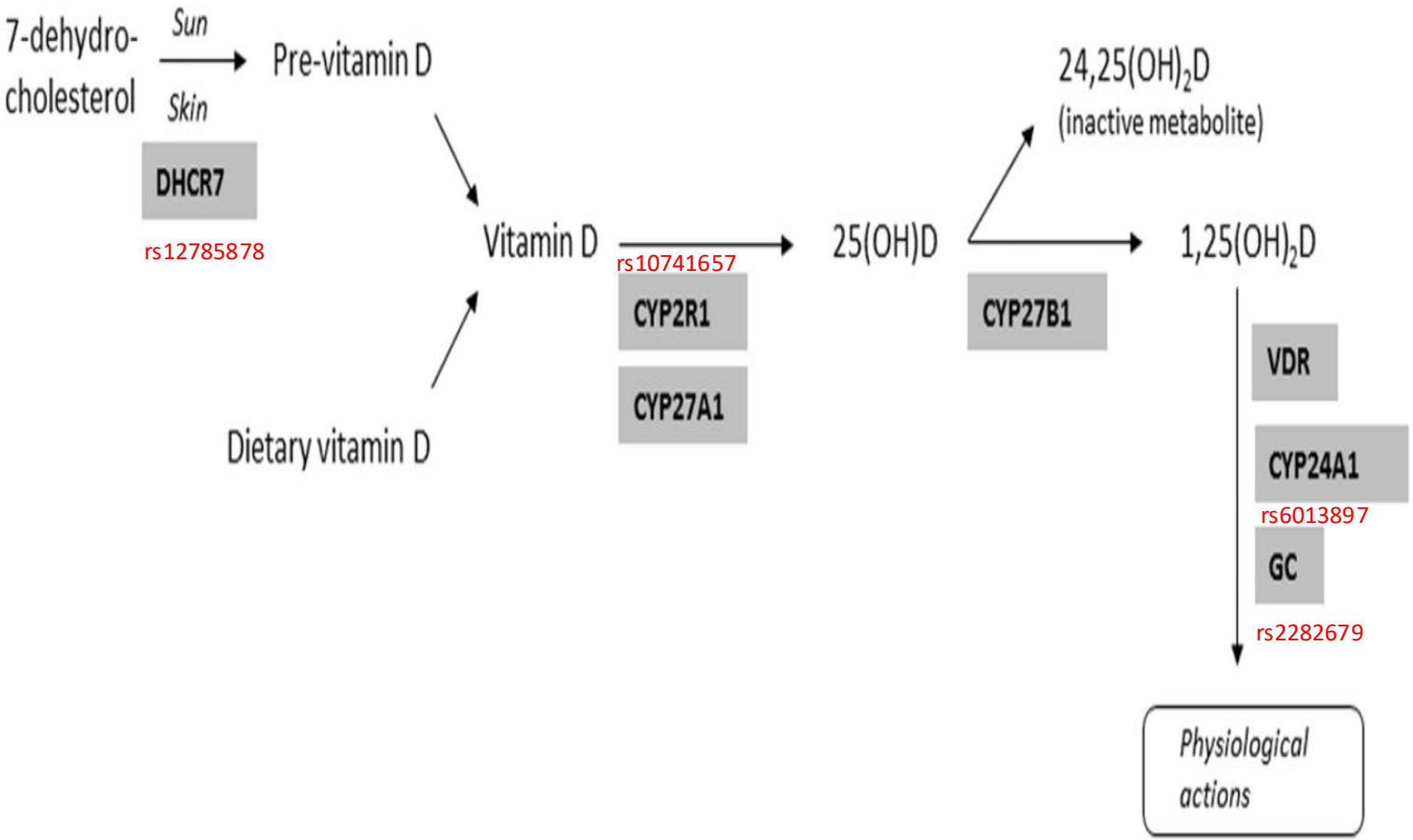
Large genetic epidemiology networks (the Genetic Associations and Mechanisms in Oncology (GAME-ON), the Genetic and Epidemiology of Colorectal Cancer Consortium (GECCO), and the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortiums, and the MR-Base platform).

of the associations with specific polymorphisms and a likelihood based approach. Secondary outcomes based on cancer subtypes by sex, anatomic location, stage, and histology were also examined.

RESULTS

There was little evidence that the multi-polymorphism score of 25(OH)D was associated with risk of any of the seven cancers or their subtypes. Specifically, the odds ratios per 25 nmol/L increase in genetically determined 25(OH)D concentrations were 0.92 (95% confidence interval 0.76 to 1.10) for colorectal cancer, 1.05 (0.89 to 1.24) for breast cancer, 0.89 (0.77 to 1.02) for prostate cancer, and 1.03 (0.87 to 1.23) for lung cancer. The results were consistent

Genetic variants associated with vitamin D concentrations

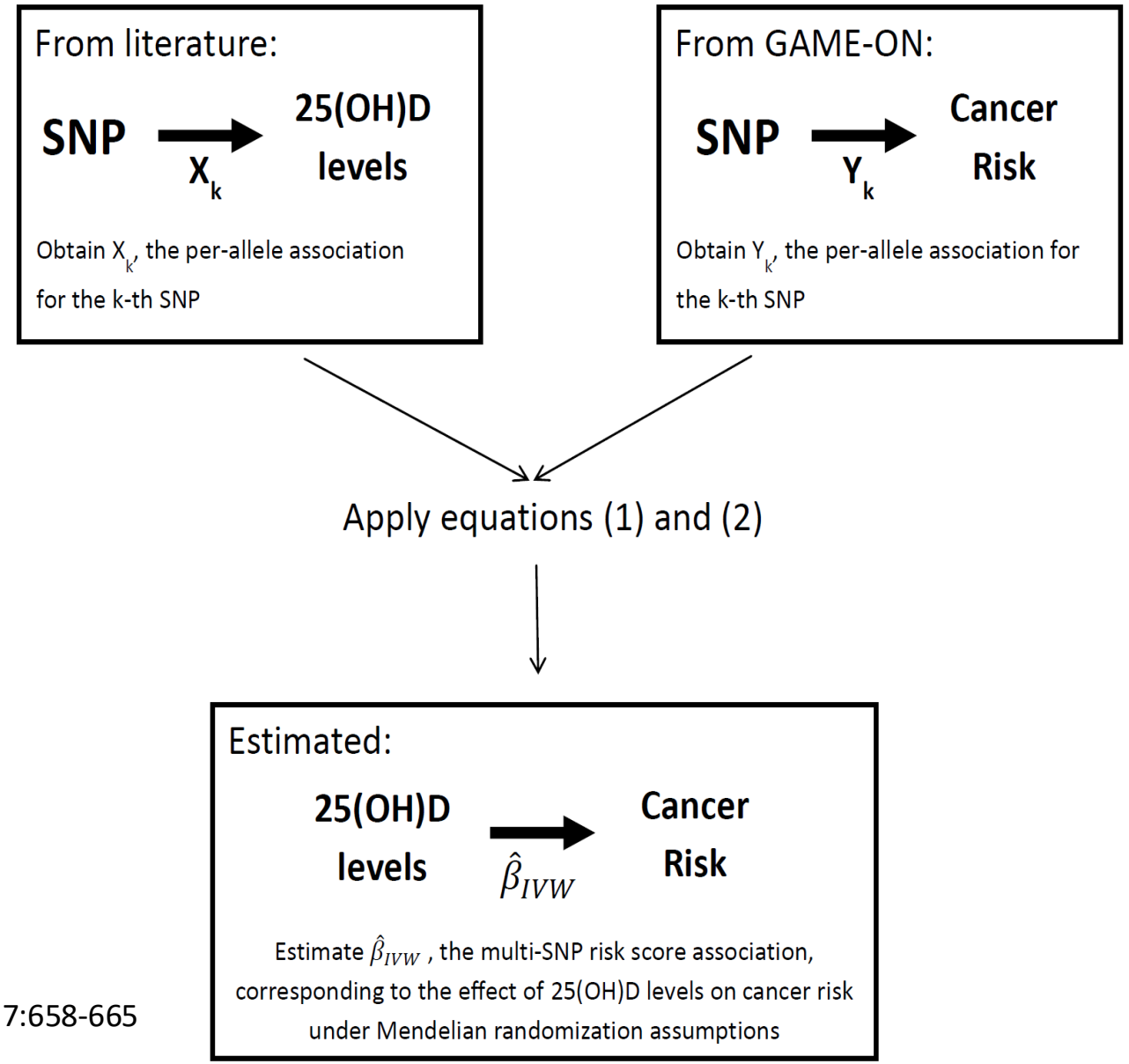


Theodoratou E, et al. Plos One 2012

Schematic of IV analysis

$$\hat{\beta}_{IVW} = \frac{\sum_k X_k Y_k \sigma_{Y_k}^{-2}}{\sum_k X_k^2 \sigma_{Y_k}^{-2}} \quad (1)$$

$$se(\hat{\beta}_{IVW}) = \sqrt{\frac{1}{\sum_k X_k^2 \sigma_{Y_k}^{-2}}} \quad (2)$$



Burgess S, et al. Genet Epidemiol 2013;37:658-665

Results

Table 3 | Mendelian randomisation estimates between multi-single nucleotide polymorphism risk scores of continuous 25(OH)D and risk of cancer calculated with inverse variance weighted method and likelihood method

		OR* (95% CI); P value	
Cancer type	**Study**	**Inverse variance weighted**	**Likelihood**
Colorectal			
All	GAME-ON	1.04 (0.78 to 1.38); 0.81	1.04 (0.78 to 1.38); 0.81
All	GECCO	0.92 (0.76 to 1.10); 0.36	0.92 (0.76 to 1.10); 0.36
All (women)	GECCO	0.92 (0.71 to 1.18); 0.52	0.92 (0.71 to 1.18); 0.52
All (men)	GECCO	0.91 (0.70 to 1.20); 0.52	0.91 (0.70 to 1.20); 0.52
Colon	GECCO	0.90 (0.73 to 1.11); 0.33	0.90 (0.73 to 1.11); 0.33
Rectal	GECCO	0.93 (0.68 to 1.26); 0.64	0.93 (0.68 to 1.26); 0.64
Distal colon	GECCO	0.97 (0.73 to 1.28); 0.83	0.97 (0.73 to 1.28); 0.83
Proximal colon	GECCO	0.83 (0.64 to 1.07); 0.14	0.82 (0.64 to 1.07); 0.14
Breast			
All	DRIVE	1.05 (0.89 to 1.24); 0.59	1.05 (0.89 to 1.24); 0.59
ER–	DRIVE	1.15 (0.88 to 1.50); 0.30	1.15 (0.88 to 1.50); 0.30
Prostate			
All	PRACTICAL	0.89 (0.77 to 1.02); 0.08	0.89 (0.77 to 1.02); 0.08
All	GAME-ON	1.08 (0.88 to 1.33); 0.47	1.08 (0.88 to 1.33); 0.46
Aggressive	GAME-ON	1.14 (0.85 to 1.54); 0.38	1.15 (0.85 to 1.54); 0.38
Ovarian			
All	FOCI	1.12 (0.86 to 1.47); 0.40	1.12 (0.86 to 1.47); 0.40
Clear-cell	FOCI	0.99 (0.46 to 2.11); 0.98	0.99 (0.46 to 2.11); 0.98
Endometrioid	FOCI	0.83 (0.48 to 1.43); 0.51	0.83 (0.48 to 1.43); 0.51
Serous	FOCI	1.26 (0.91 to 1.76); 0.17	1.26 (0.91 to 1.76); 0.17
Lung			
All	TRICL-ILCCO	1.03 (0.87 to 1.23); 0.72	1.03 (0.87 to 1.23); 0.72
Adenocarcinoma	TRICL-ILCCO	1.03 (0.79 to 1.35); 0.84	1.03 (0.79 to 1.35); 0.84
Squamous	TRICL-ILCCO	0.95 (0.72 to 1.25); 0.74	0.95 (0.72 to 1.25); 0.74
Pancreatic			
All	PanScan1†	1.36 (0.81 to 2.27); 0.25	1.36 (0.80 to 2.27); 0.25
Neuroblastoma			
All	Capasso, et al[17]†	0.76 (0.47 to 1.21); 0.24	0.76 (0.47 to 1.21); 0.24

Dimitrakopoulou V & Tsilidis KK, et al. BMJ 2017

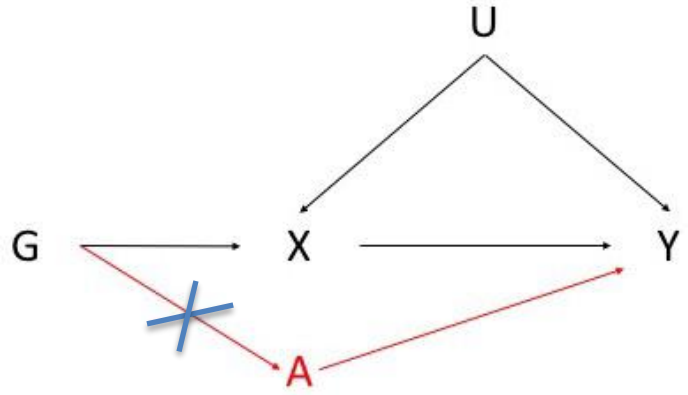
Discussion

- A multi-SNP score for 25(OH)D was not associated with risk of seven cancers.
- IV assumptions don't seem violated, although we cannot prove their validity.
- Limitations due to use of summary data
  - Cannot perform stratified analyses
  - Assumed linear associations
  - Cannot more fully assess IV assumptions
- Other limitations
  - Small fraction of 25(OH)D variation explained by the three used SNPs?
  - Potential pleiotropy of used SNPs?

Methods to assess MR assumptions

- Horizontal pleiotropy is the most problematic assumption
  - Function of genetic variants is often unknown, particularly if these have been identified in GWAS

- We do have methods available to deal with pleiotropy



Methods to assess pleiotropy

- In one-sample MR, very few methods available to detect (Sargan test) and correct for pleiotropy

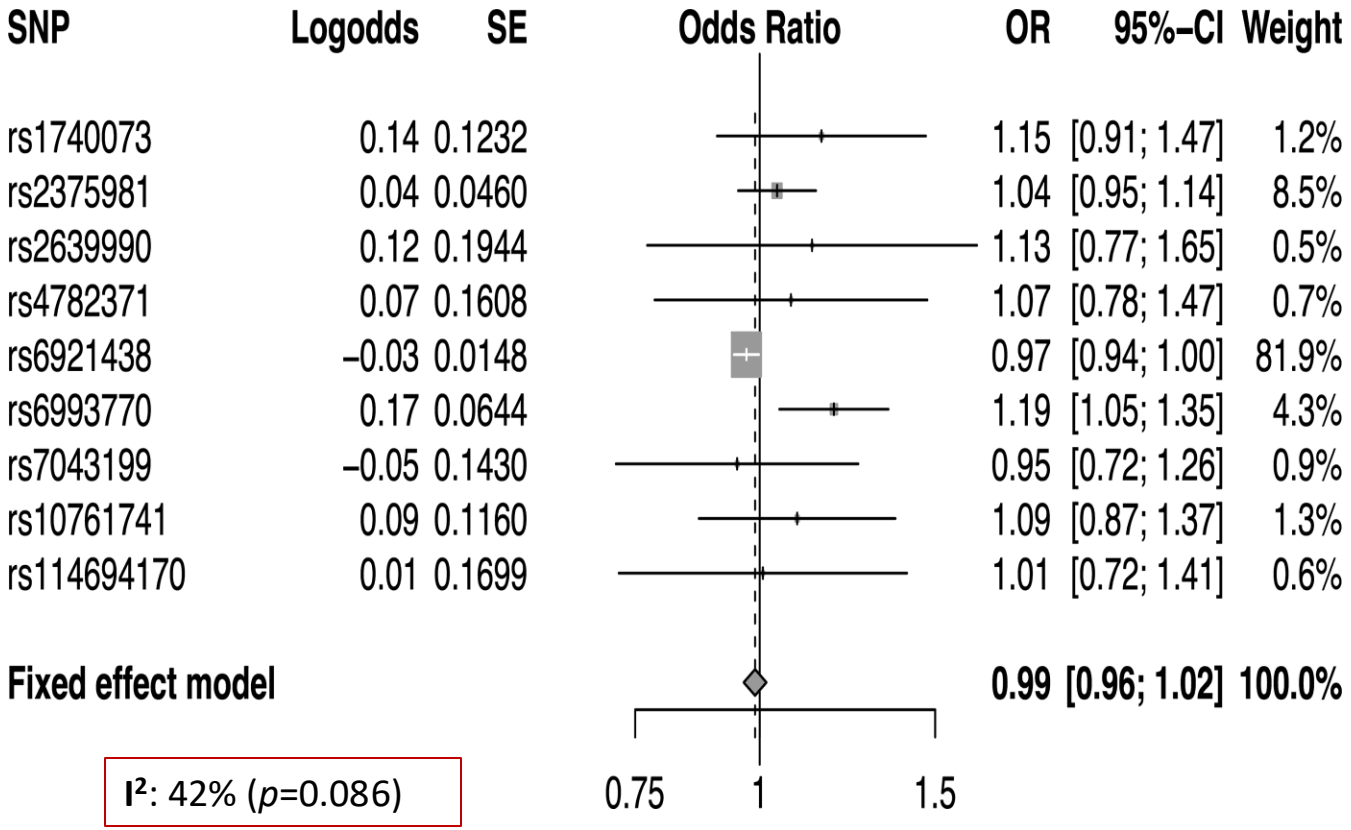
- In two-sample MR, can detect pleiotropy with:
  - Cochran's Q test
  - I^2 statistic
  - Diagnostic plots (e.g. forest, funnel plots)
  - MR-Egger intercept test

- In two-sample MR, can correct for pleiotropy with:
  - MR-Egger slope
  - Weighted median
  - MR PRESSO
  - Several more…

Glymour MM, et al. Am J Epidemiol 2012;175:332-9.
Bowden J, et al. Int J Epidemiol 2015;44:512-25.
Bowden J, et al. Genet Epidemiol 2016;40:304-14.
Greco et al. Stat Med 2017

Methods to detect pleiotropy

Example:
Forest plot of
MR estimates



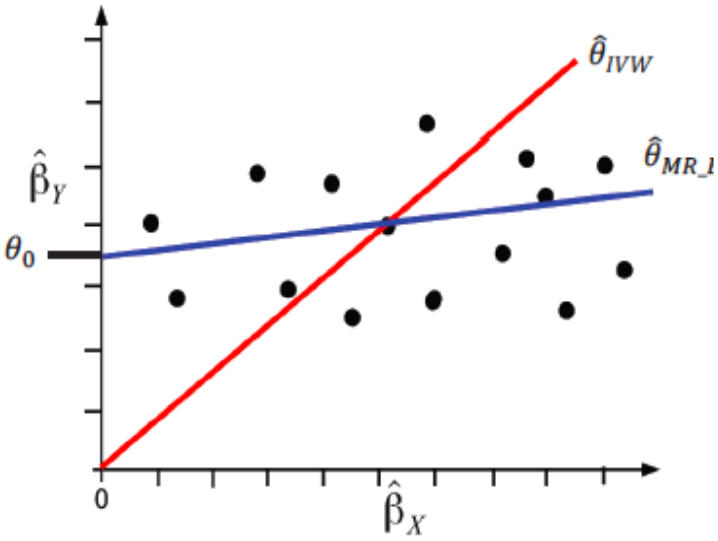
Au Yeung SL et al. Vascular Endothelial Growth Factor and Ischemic Heart Disease Risk: A Mendelian Randomization Study. J Am Heart Assoc; 2017;6:e005619

MR-Egger regression

- MR-Egger relaxes the assumption that the average pleiotropic effect is zero (to allow for directional pleiotropy)
- This is achieved by introducing an intercept θ_0 in the regression model:

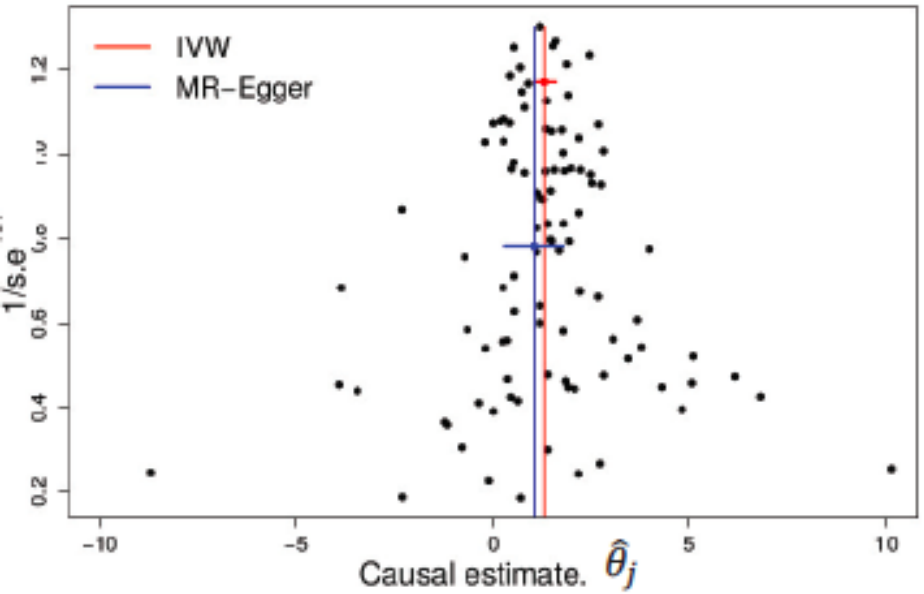
$$\hat{\beta}_{Yj} = \theta_0 + \theta\,\hat{\beta}_{Xj} + \varepsilon_j$$

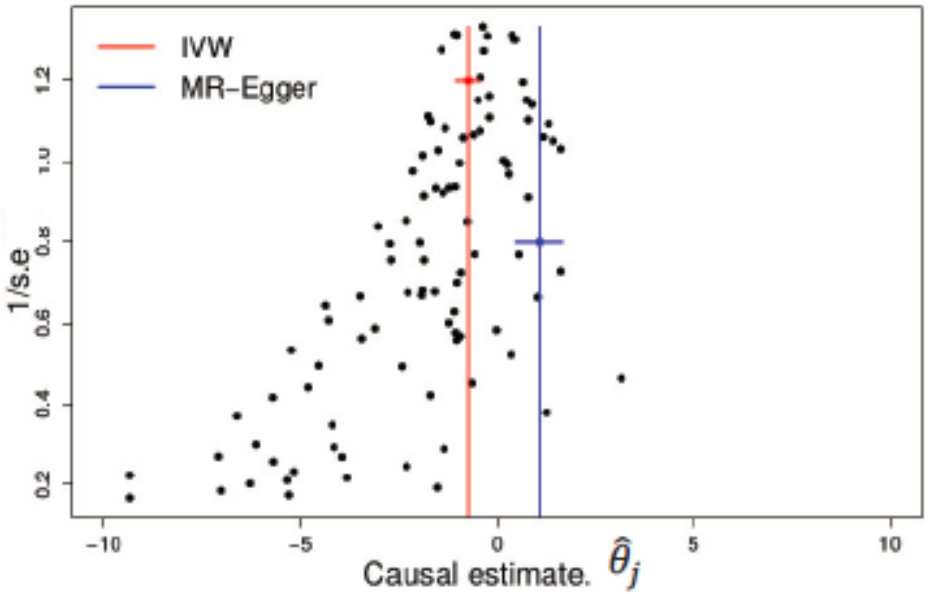
- The intercept should be zero if all variants are valid IVs
- θ is the MR-Egger causal effect



Bowden J, et al. Int J Epidemiol 2015:512-25

MR-Egger regression: examples





- Left: funnel appears symmetric, i.e. balanced pleiotropy appears valid, and IVW suitable
- Right: funnel is asymmetric, i.e. directional pleiotropy probable, and IVW not suitable

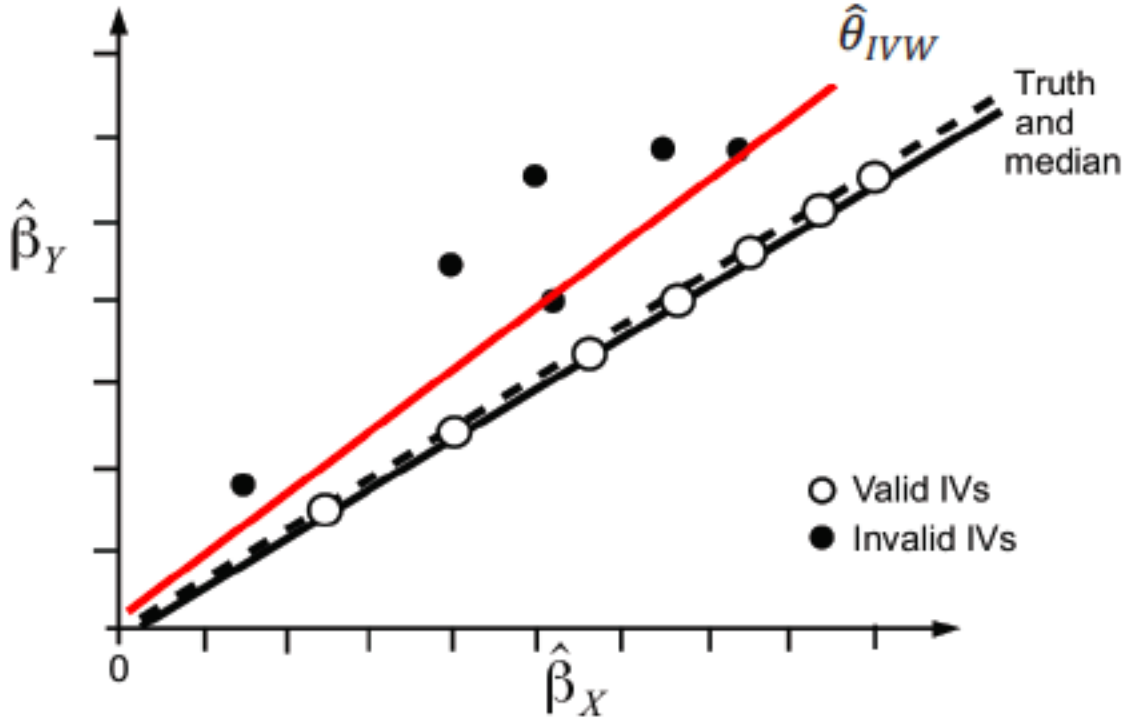
Bowden J, et al. Int J Epidemiol 2015:512-25

Median based estimation

- Suppose that the majority of variants, i.e. >50%, are valid IVs
- In a large sample size, the variant-specific ratio estimates based on the valid instruments will all estimate the true causal effect
- So the median of the ratio estimates can be used as an estimate of θ
- The median estimate will be less influenced by outlying variants than the IVW estimate (which is a weighted mean of the variant-specific ratio estimates)
- No assumption necessary for invalid variants

Bowden J, et al. Gen Epidemiol 2016;40:304-14

Example: infinite sample data



- Artificial example: 7 valid IVs, 6 invalid
- With infinite data, only the IVW estimate is biased

Bowden J, et al. Gen Epidemiol 2016;40:304-14

Comparison of robust MR methods

Method	% invalid IVs	Violation of 2nd assumpt allowed	Violation of 3rd assumpt allowed	Strengths	Weaknesses
IVW	0%	No	No	-Efficient	-Sensitive to invalid IVs -Sensitive to outliers
MR-Egger	100%	No	Yes	-Consistent with invalid IVs	-InSIDE assumption -Less efficient -Sensitive to outliers
Weighted median	50%	Yes	Yes	-Consistent when <50% of weight contributed by invalid IVs -Robust to outliers	-Less efficient than IVW (more efficient than MR-Egger)

Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO)

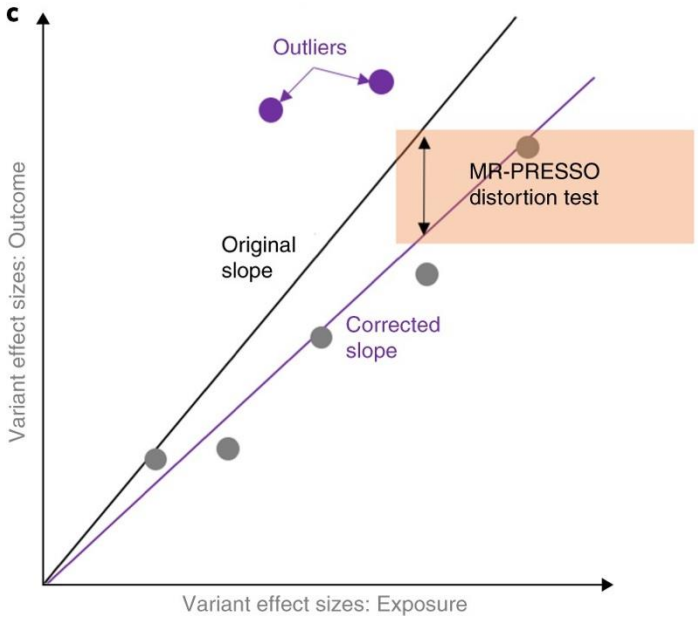
- MR-PRESSO test aims to evaluate horizontal pleiotropy in multi-instrument summary-level MR.

MR-PRESSO has three components:

1. Detection of horizontal pleiotropy (the MR-PRESSO global test)

2. Correction for horizontal pleiotropy via outlier removal (the MR-PRESSO local test for outliers)

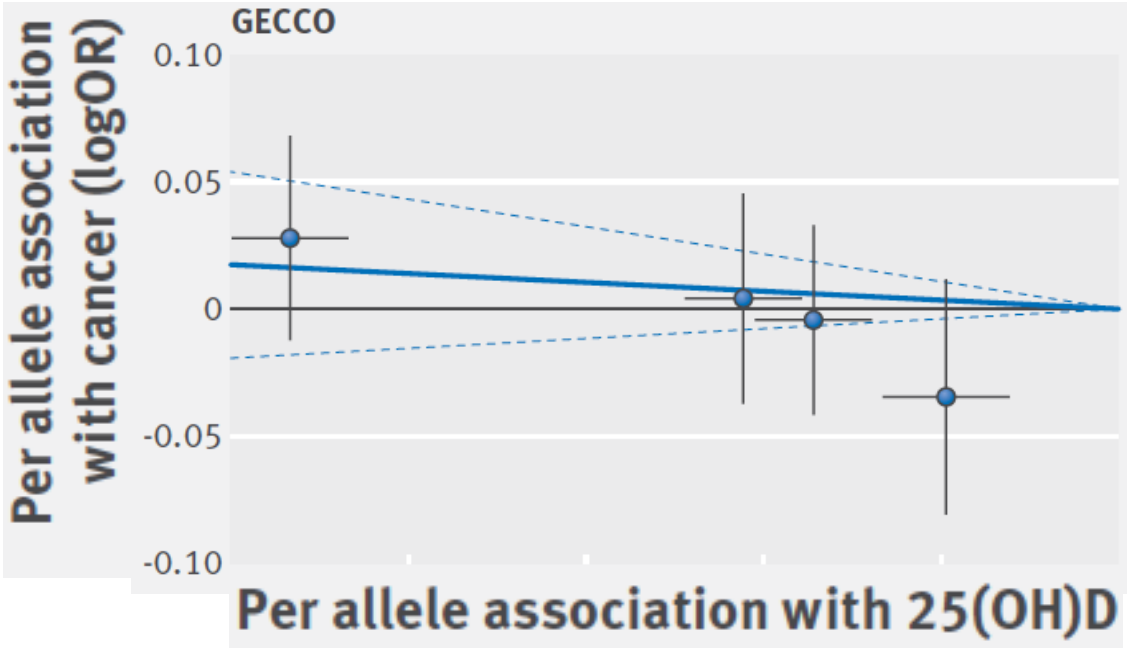
3. Testing of significant differences in the causal estimates before and after correction for outliers (the MR-PRESSO distortion test)



Verbanck M, et al. Nat Gen 2018

Example: Circulating vitamin D concentrations (25(OH)D) and colorectal cancer risk

- 4 IVs; approx. 11,500 case-control pairs
- $OR_{IVWfixed}$: 0.92 (0.76 – 1.10)
- $OR_{MR\text{-}Egger}$: 0.70 (0.49 – 1.02); $P_{intercept}$: 0.25
- $OR_{weighted\ median}$: 0.89 (0.73 – 1.08)



Dimitrakopoulou V & Tsilidis KK, et al. BMJ 2017

Example: Coffee consumption and breast cancer risk

- 33 IVs; approx. 120,000 case-control pairs
- $OR_{IVWfixed}$: 0.91 (0.86 – 0.95); P_{het}:10^{-13}
- $OR_{IVWrandom}$: 0.91 (0.80 – 1.02)
- $OR_{MR\text{-}Egger}$: 1.00 (0.80 – 1.25); $P_{intercept}$: 0.30
- $OR_{weighted\ median}$: 0.97 (0.89 – 1.05)
- $OR_{weighted\ mode}$: 1.00 (0.93 – 1.07)

Ellingjord-Dale M, et al. Plos One 2021

Example: Magnesium concentrations and breast cancer risk

- 6 IVs; approx. 120,000 case-control pairs
- $OR_{IVWfixed}$: 1.17 (1.10 – 1.25); P_{het}:0.40
- $OR_{IVWrandom}$: 1.17 (1.10 – 1.25)
- $OR_{MR\text{-}Egger}$: 1.24 (1.01 – 1.53); $P_{intercept}$: 0.57
- $OR_{weighted\ median}$: 1.20 (1.10 – 1.31)
- $OR_{weighted\ mode}$: 1.21 (1.12 – 1.39)

Papadimitriou N, et al. Int J Cancer 2021

Summary of methods to assess pleiotropy

- We have discussed various methods that can be used to assess the robustness of a MR analysis
- The aim is not to recommend a single authoritative method for all analyses
- Rather, examining the results from different methods that make different assumptions (i.e. IVW, MR-Egger, weighted median, etc.) provides a sensitivity analysis that either adds to or questions the robustness of a finding from a MR investigation
- Causal inferences are more plausible when there are consistent findings across methods making different assumptions

Limitations and promise of MR studies

- Sometimes…
  - Lack of suitable polymorphisms for studying modifiable exposures
  - Failure to establish reliable associations between genotype-exposure and genotype-disease due to limited sample sizes
  - Confounding due to linkage disequilibrium and population stratification
  - Pleiotropy and multi-functionality of genes
- Need large sample sizes (because gene variants typically yield only small changes in exposure variable)
- Need replication!
- **But, great potential of MR to assist causal inference in the future given large samples from genetic consortia, new efficient study design methods and new methods for testing MR assumptions (e.g., MR Egger, weighted median, MR PRESSO, etc.)**