Mendelian Randomization

David Evans

University of Queensland
University of Bristol

Some Criticisms of GWA Studies…

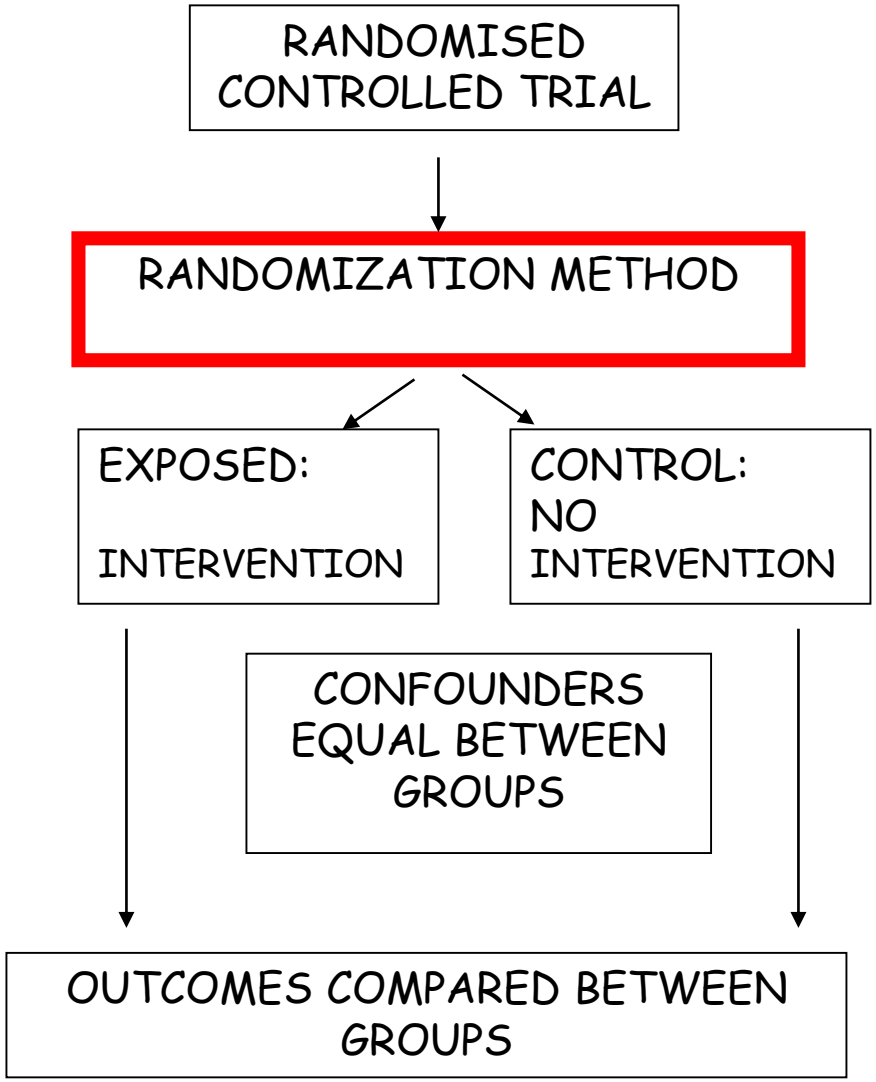
- How do you translate the results from GWAS?
- You can't change people's genotypes (at least not yet)
- You can however modify people's environments…
- Mendelian Randomization is a method of using genetics to inform us about associations in traditional observational epidemiology and MUCH MUCH more…

This Session

- Determining causality in observational studies
- Mendelian randomization (MR)
- An Example of MR
- MRBase
- MR and Drug Development
- Practical

RCTs: the Gold Standard in Inferring Causality

Randomization makes causal inference possible



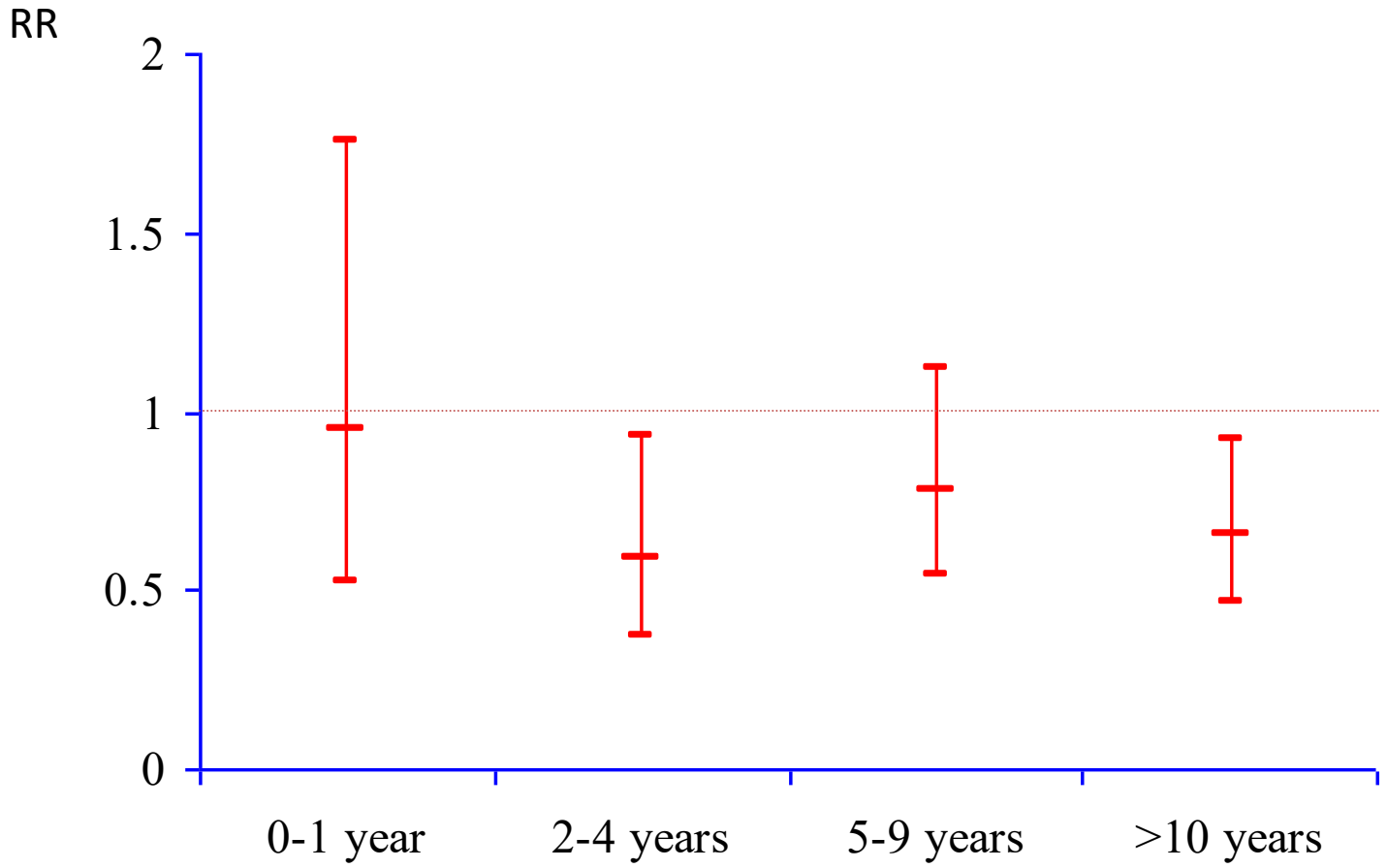
The Need for Observational Studies

- **Randomized Controlled Trials (RCTs):**
  - Not always ethical or practically feasible eg anything toxic
  - Expensive, requires experimentation in humans
  - 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)
    eg case-control studies or cohort studies
  - Reliably assigning causality in these types of studies is ***very limited***


Today's Random Medical News
from the New England Journal of Panic-Inducing Gobbledygook
JIM BORGMAN
EXERCISE
FATTY FOODS
STRESS
RED WINE
DAYCARE
COMPUTER TERMINALS
COFFEE
SMOKING
CAN CAUSE
HEART DISEASE
BREAST CANCER
SPONTANEOUS REMISSION
DEPRESSION
SEXUAL DYSFUNCTION
A FEELING OF WELL-BEING
HYPO-THERMIA
IN
CHILDREN
TWINS
ARTHRITIS SUFFERERS
7 OUT OF 10 WOMEN
RATS
OVER-WEIGHT SMOKERS
MEN 25-40
TWO-INCOME FAMILIES
ACCORDING TO A REPORT RELEASED TODAY....
NEWS

CHD risk according to duration of current Vitamin E supplement use compared to no use



Rimm et al NEJM 1993; 328: 1450-6

The New York Times
nytimes.com

May 20, 1993

Vitamin E Greatly Reduces Risk Of Heart Disease, Studies Suggest

By JANE E. BRODY

Two new studies of more than 120,000 men and women strongly suggest that supplements of vitamin E can significantly reduce the risk of dise researchers and other experts cautioned against rushing out to buy the vitamin supplements before further clinical trials confirm that they are be

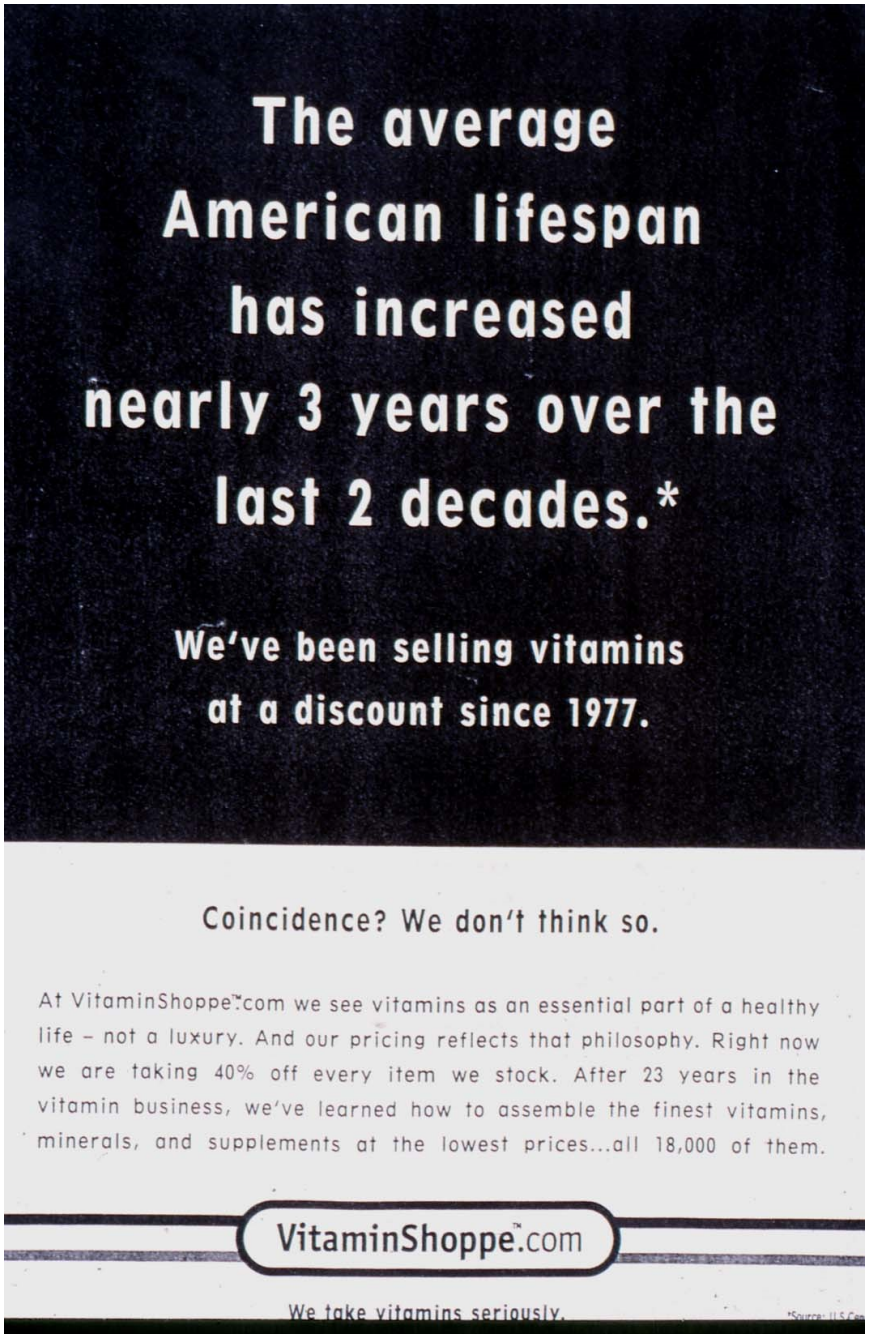
The studies, by researchers at the Harvard School of Public Health and Brigham and Women's Hospital in Boston, showed that initially healthy coronary disease at a rate about 40 percent lower than comparable men and women whose intake of this vitamin was lowest. The preventive e blood levels of cholesterol.

The greatest protection was found at levels of about 100 international units of vitamin E a day for more than two years. The Federal recommen consume fewer than 25 units from foods like vegetable oils, wheat germ, seeds, whole grains and nuts.

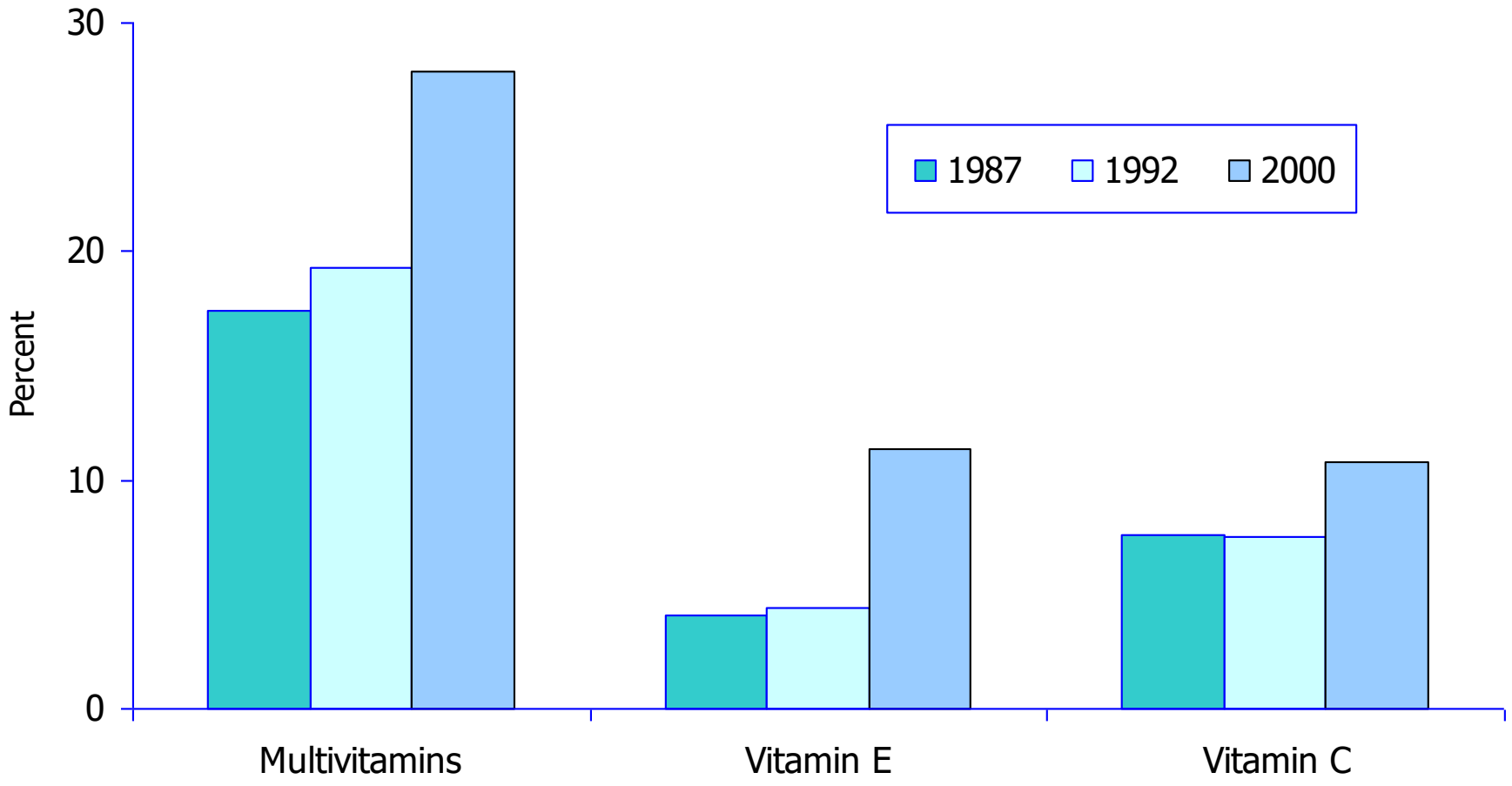
The researchers said vitamin E, as an antioxidant, might reduce heart disease by having an effect on low-density lipoprotein cholesterol, or LDL type of cholesterol damages arteries primarily after it has been oxidized.

The new findings, which appear today in The New England Journal of Medicine, are some of the first to find health benefits from taking large-d "megadoses" of vitamins as a popular remedy whose value is unproven. Expert Urge Caution

While a person might conclude from the findings that it would be wise to take large doses of vitamin E supplements daily, their long-term safety

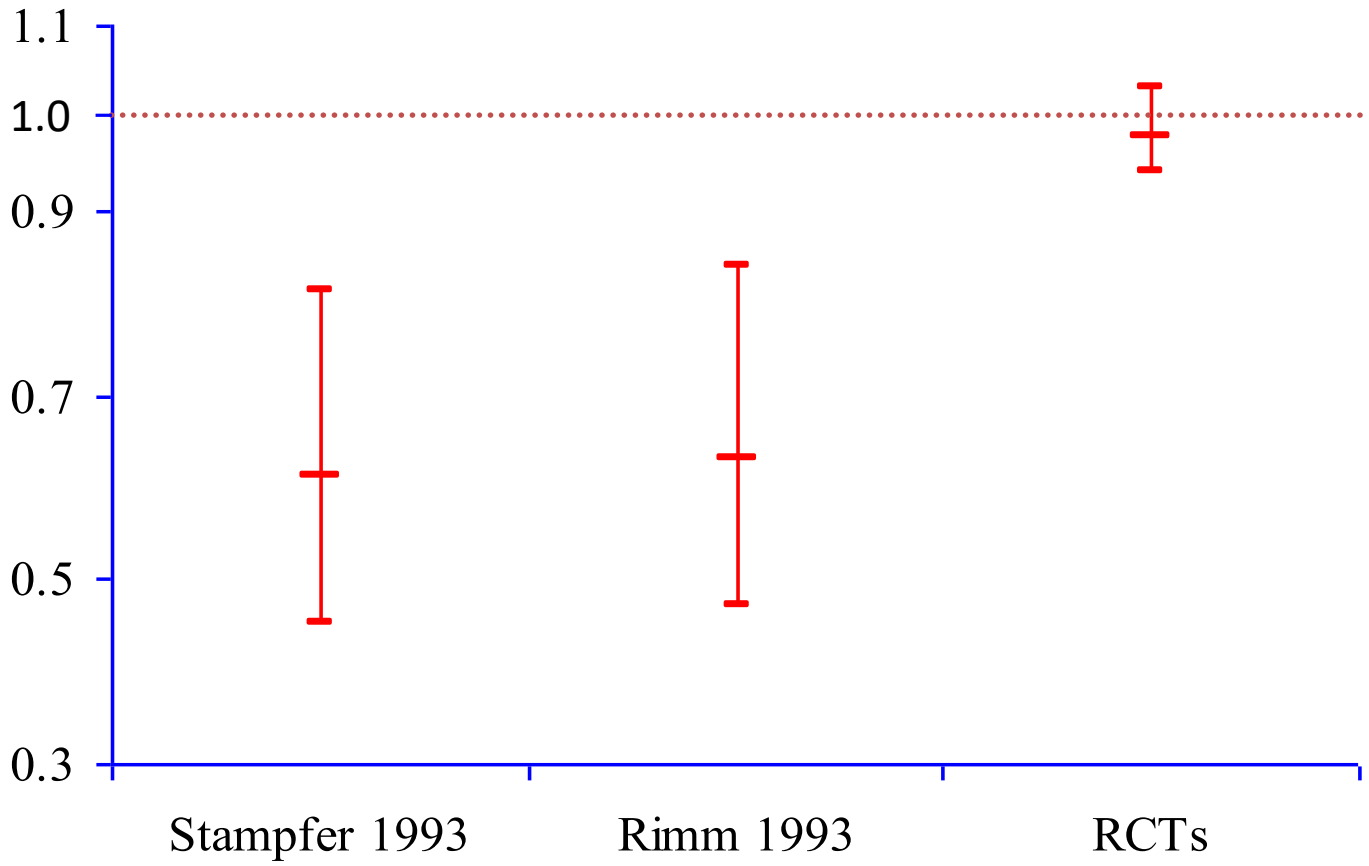

The average American lifespan has increased nearly 3 years over the last 2 decades.*
We've been selling vitamins at a discount since 1977.
Coincidence? We don't think so.
At VitaminShoppe™.com we see vitamins as an essential part of a healthy life – not a luxury. And our pricing reflects that philosophy. Right now we are taking 40% off every item we stock. After 23 years in the vitamin business, we've learned how to assemble the finest vitamins, minerals, and supplements at the lowest prices...all 18,000 of them.
VitaminShoppe™.com
We take vitamins seriously.

Use of vitamin supplements by US adults, 1987-2000

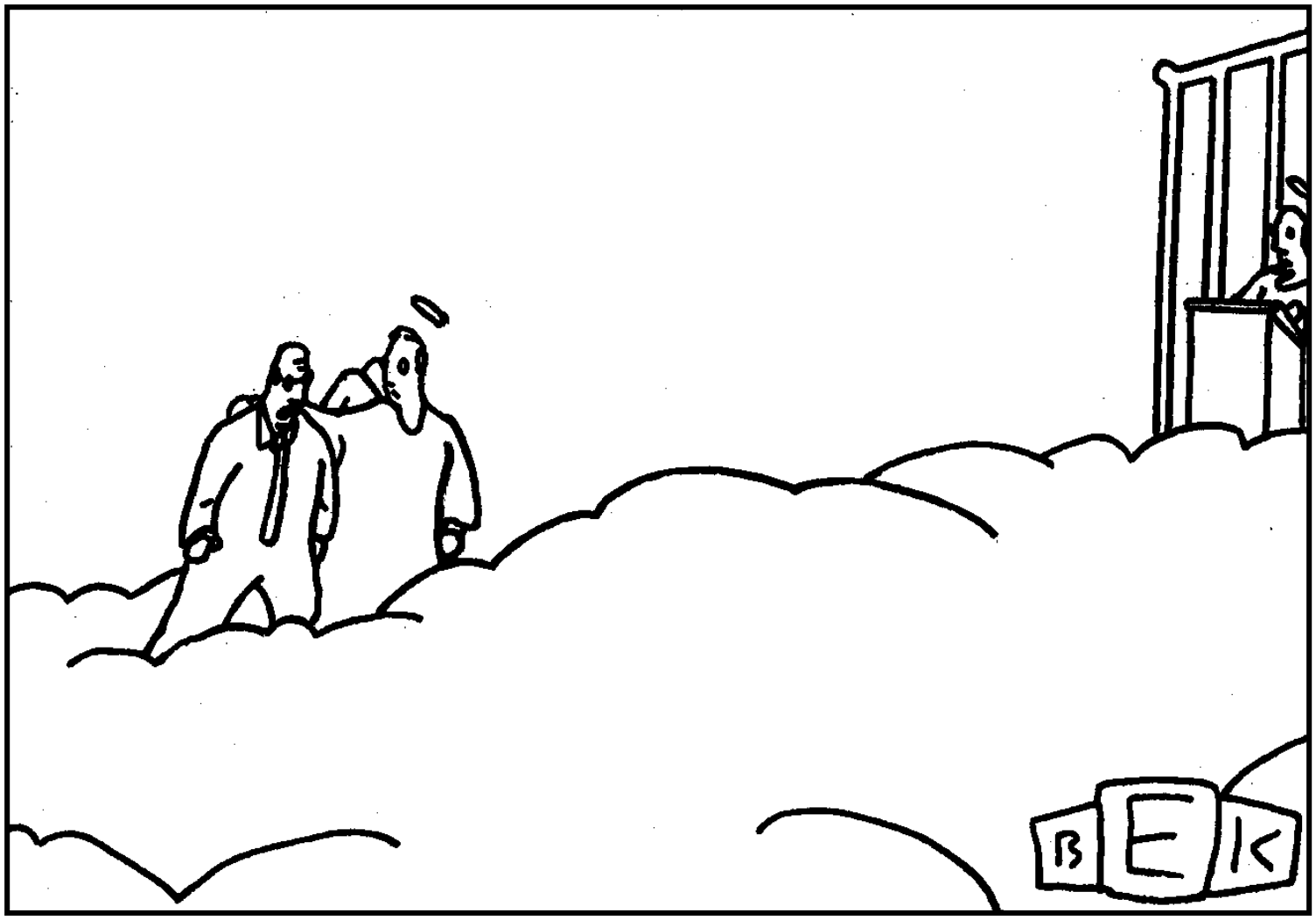


Source: Millen AE, Journal of American Dietetic Assoc 2004;104:942-950

Vitamin E supplement use and risk of Coronary Heart Disease



Stampfer et al NEJM 1993; 328: 144-9; Rimm et al NEJM 1993; 328: 1450-6; Eidelman et al Arch Intern Med 2004; 164:1552-6



“Well, so much for antioxidants.”

MANY OTHER EXAMPLES

VITAMIN C, VITAMIN A, HRT,
MANY DRUG TARGETS…….

WHAT'S THE EXPLANATION?

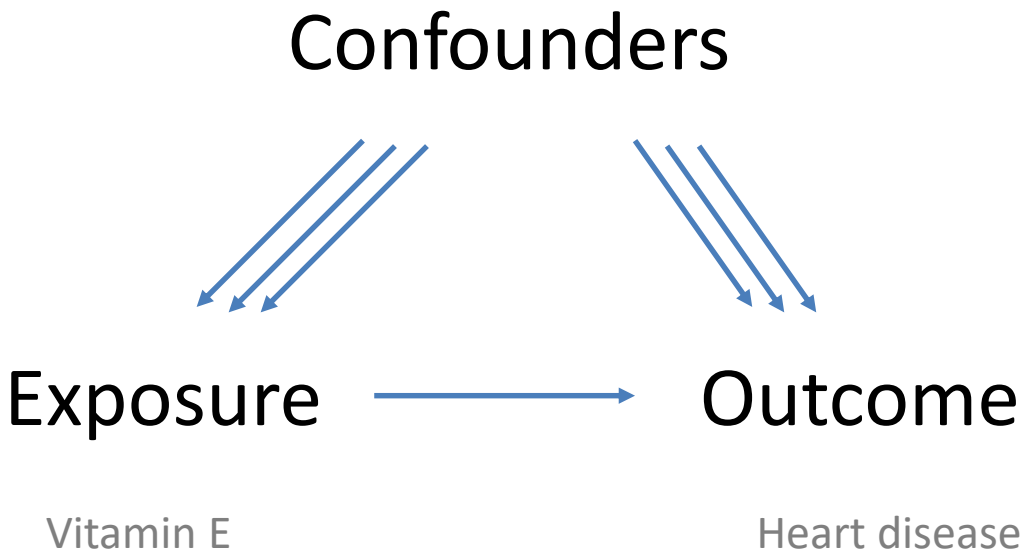
Vitamin E levels and confounding risk factors:

Childhood SES	↓
Manual social class	↓
No car access	↓
State pension only	↓
Smoker	↓
Obese	↓
Daily alcohol	↑
Exercise	↑
Low fat diet	↑
Height	↑
Leg length	↑

Women's Heart and Health Study
Lawlor et al, Lancet 2004

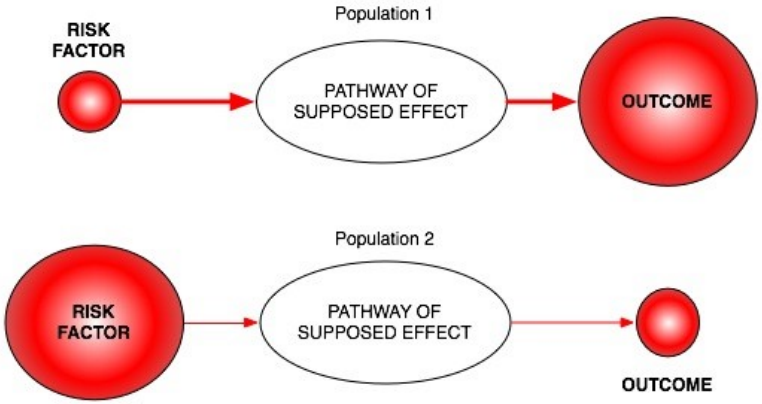
Confounding

Smoking, diet, alcohol, socioeconomic position….



Classic limitations to “observational” science

- **Confounding**
- **Reverse Causation**
- Bias



Mendelian randomization

How can it help
observational epidemiology?

What is Mendelian randomization?

- Mendelian randomization (MR) is an epidemiological technique that uses genetic variants as proxy measures for an environmental exposure

- It is an application of "Instrumental Variable" (IV) analysis:
  - it uses genetic variants as 'instruments' for the exposure of interest. An IV is a variable that is only associated with an outcome because of its association with the exposure

What does MR do?

- **Assess causal relationship between two variables**

- Estimate magnitude of causal effect

How does it do this?

By harnessing Mendel's laws of inheritance

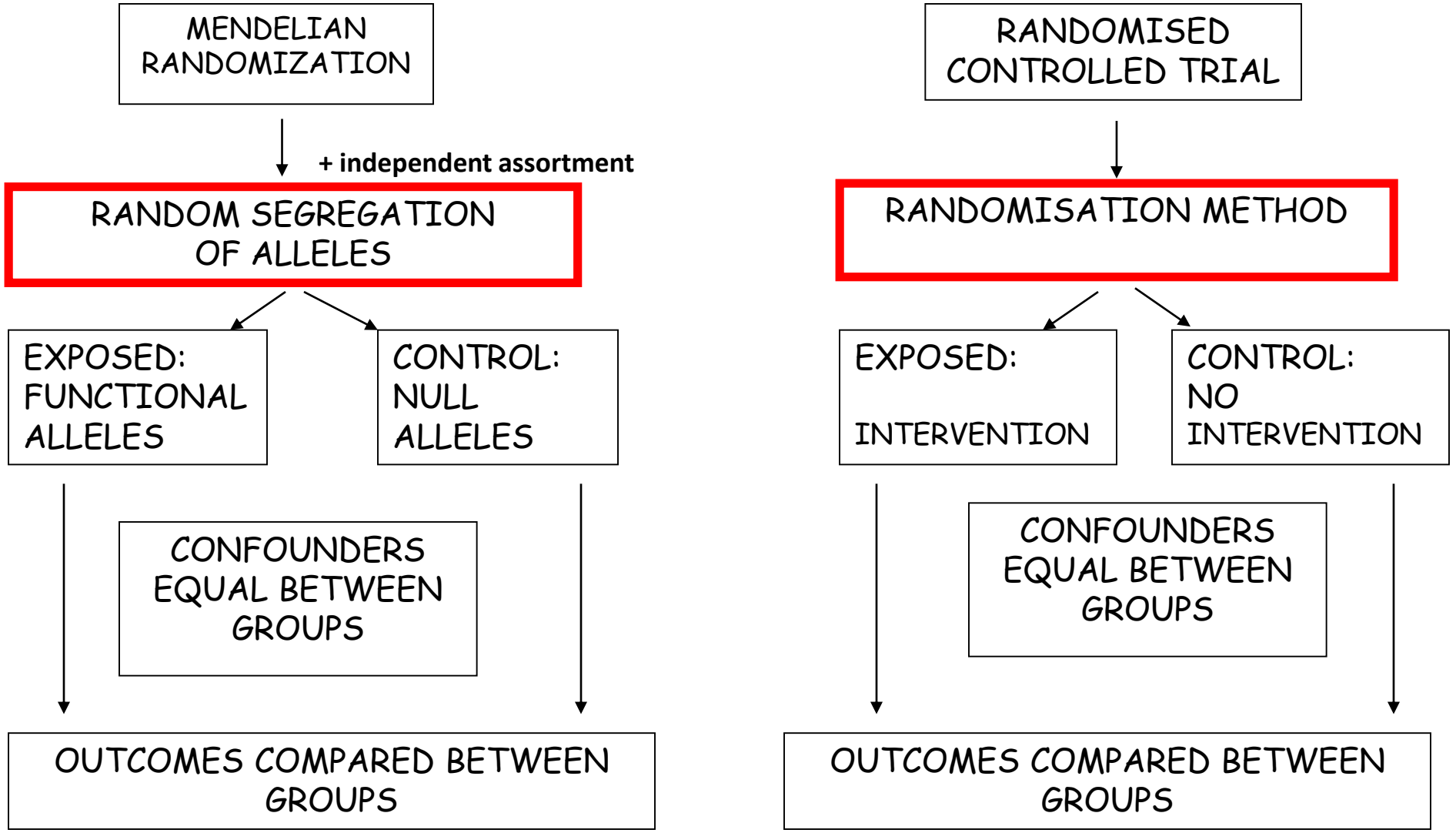
Mendel's Laws of Inheritance

Mendel in 1862

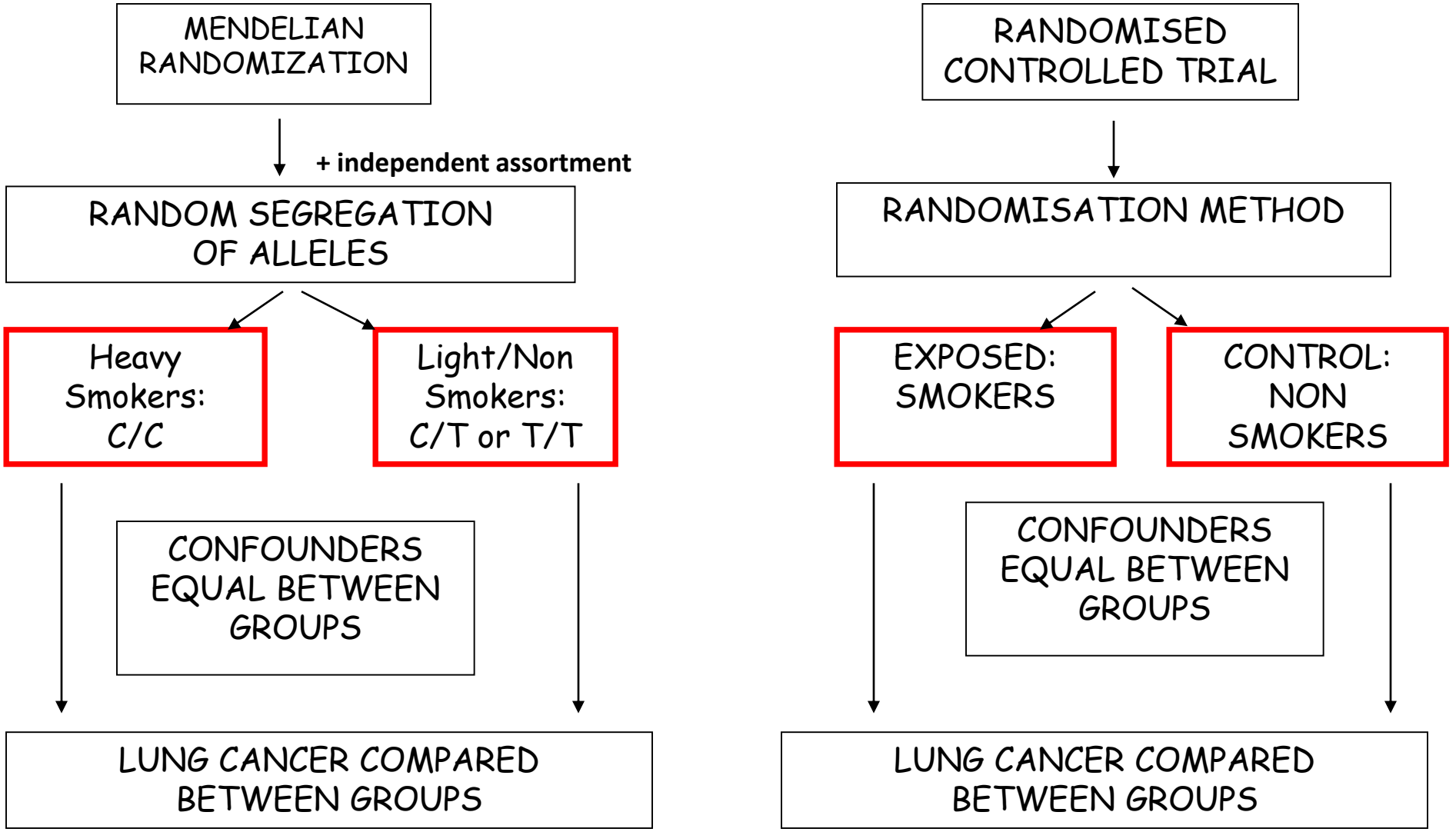
1. **Segregation:** alleles separate at meiosis and a randomly selected allele is transmitted to offspring

2. **Independent assortment:** alleles for separate traits are transmitted independently of one another

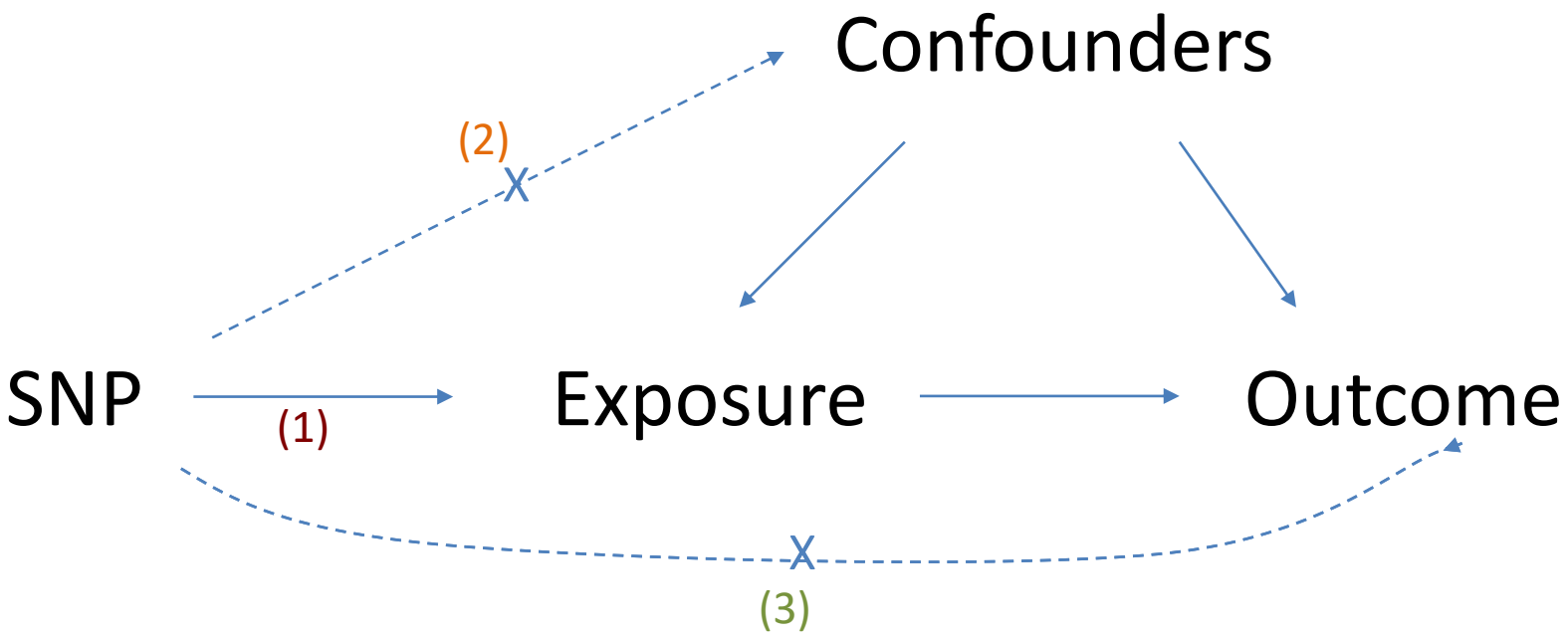
Mendelian randomization and RCTs



Mendelian randomization: Smoking and Lung Cancer



Mendelian Randomization: 3 Core Assumptions

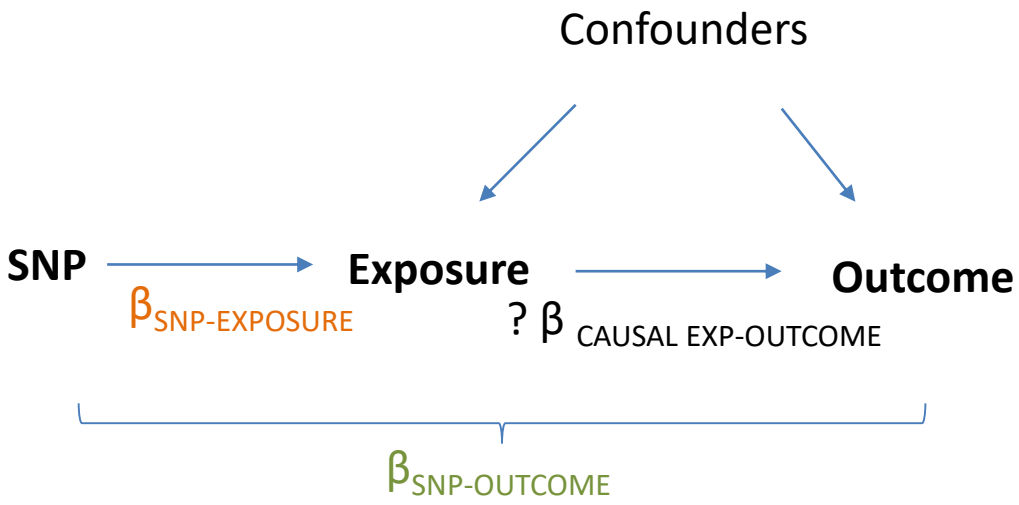


(1) SNP is associated with the exposure

(2) SNP is NOT associated with confounding variables

(3) SNP ONLY associated with outcome through the exposure

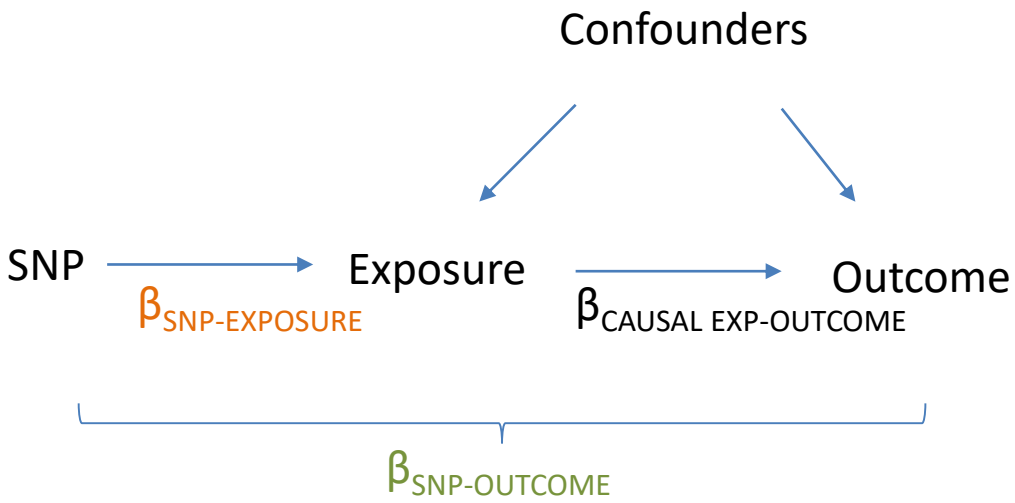
Calculating Causal Effect Estimates



After SNP identified robustly associated with exposure of interest:

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

Calculating Causal Effect Estimates



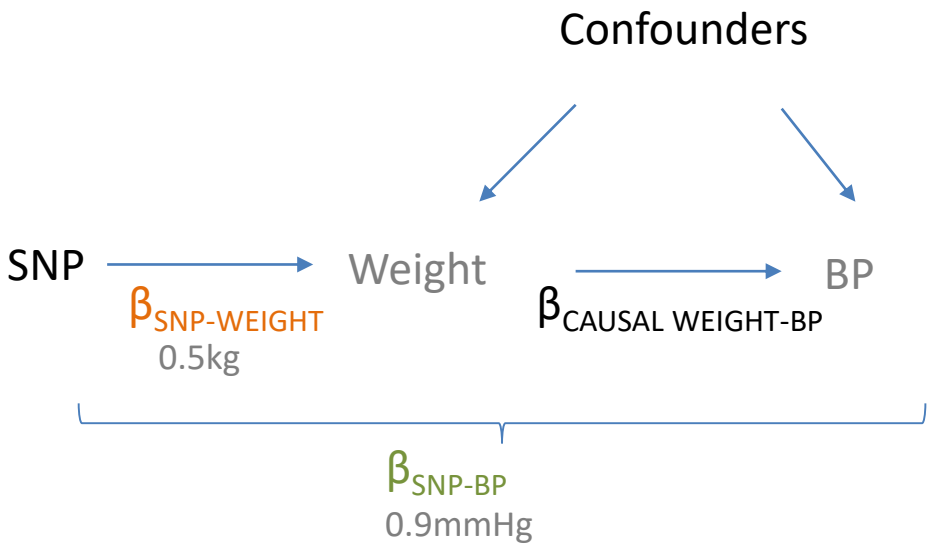
Causal effect by Wald Estimator* :

$$\frac{\beta_{SNP\text{-}OUTCOME}}{\beta_{SNP\text{-}EXPOSURE}}$$

$$\beta_{SNP\text{-}OUTCOME} = \beta_{CAUSAL\ EXP\text{-}OUTCOME} \times \beta_{SNP\text{-}EXPOSURE}$$

*Can be used in different samples ("Two sample MR")

Calculating Causal Effect Estimates



Causal effect by Wald Estimator* :

$$\frac{\beta_{SNP\text{-}OUTCOME}}{\beta_{SNP\text{-}EXPOSURE}}$$

= change in outcome per unit change in exposure

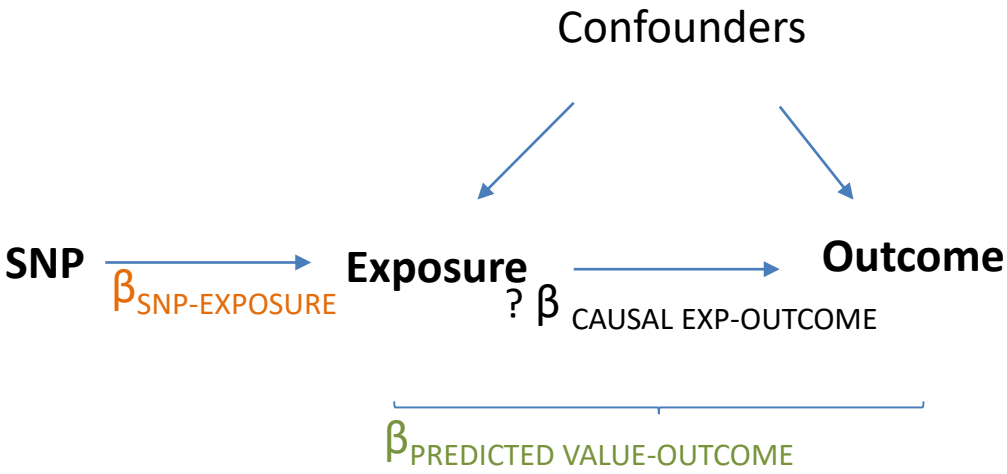
BP and weight:

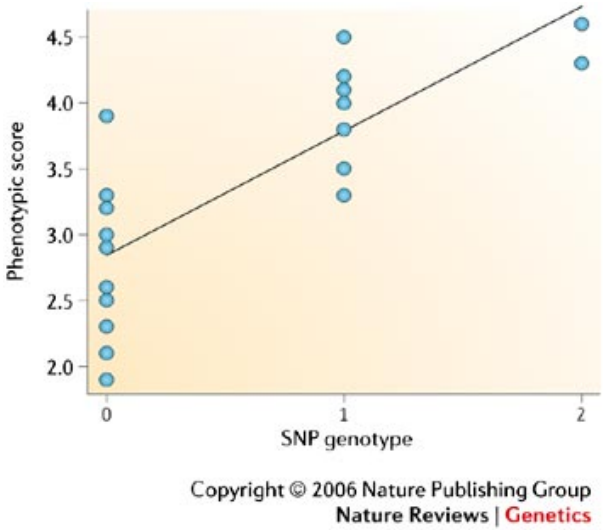
$$\frac{0.9\ \text{mmHg/allele}}{0.5\ \text{kg/allele}}$$

=1.8 mmHg/kg

*Can be used in different samples ("Two sample MR")

Calculating Causal Effect Estimates





Two-stage Least Squares (2SLS):

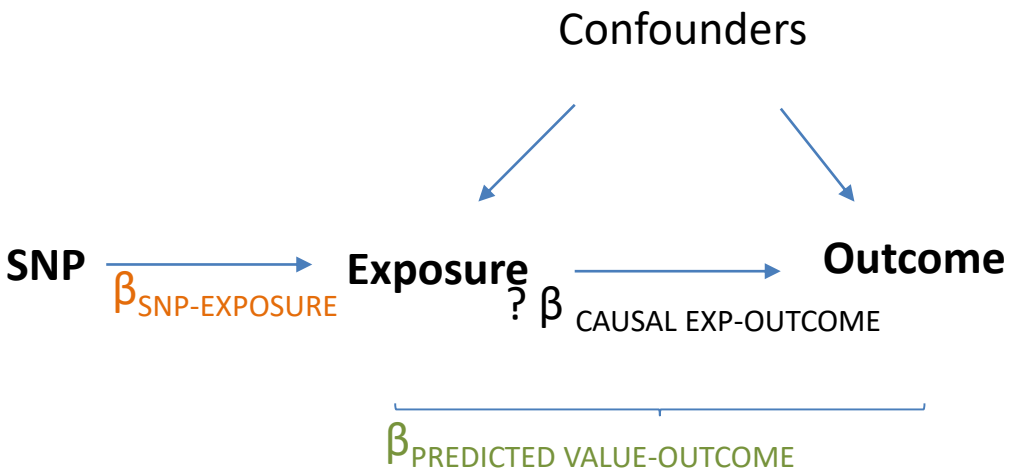
(1) Regress exposure on SNP & obtain predicted values

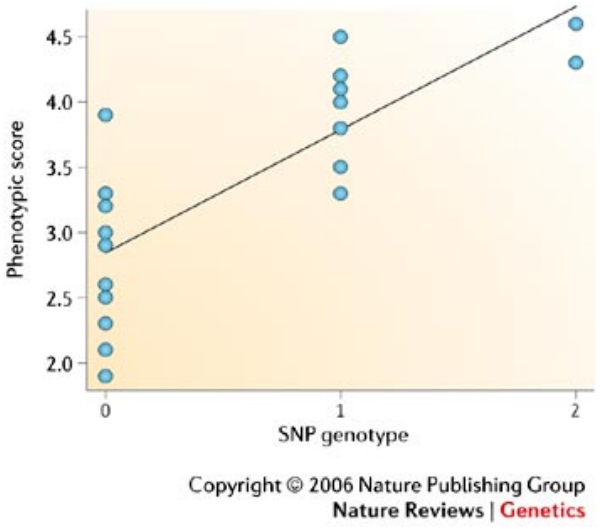
(2) Regress outcome on **predicted** exposure (from 1st stage regression)

(3) Adjust standard errors

*Needs to be done in the one sample ("Single sample MR")

Calculating Causal Effect Estimates





Two-stage Least Squares (2SLS):

(1) Regress exposure on SNP & obtain predicted values

(2) Regress outcome on **predicted** exposure (from 1st stage regression)

(3) Adjust standard errors

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

Genetically determined exposure → "randomized" → can ascribe causality

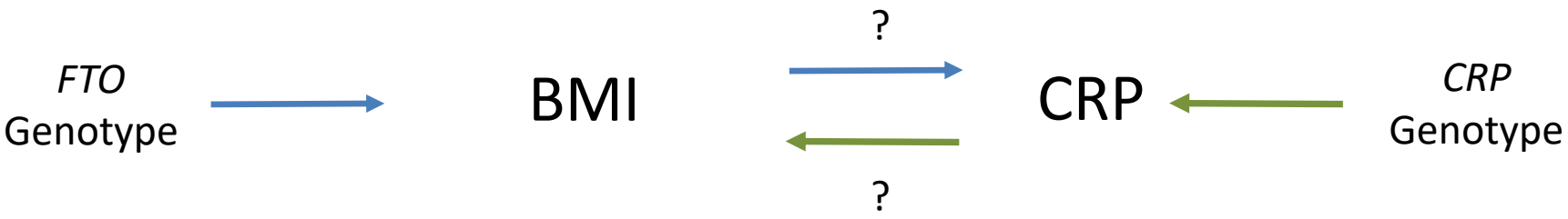
(if assumptions are met)

*Needs to be done in the one sample ("Single sample MR")

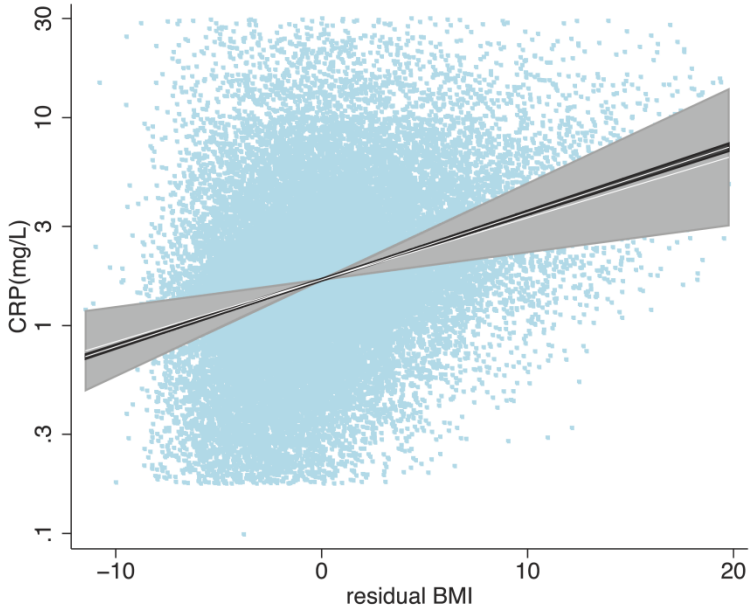
MR Example using CRP

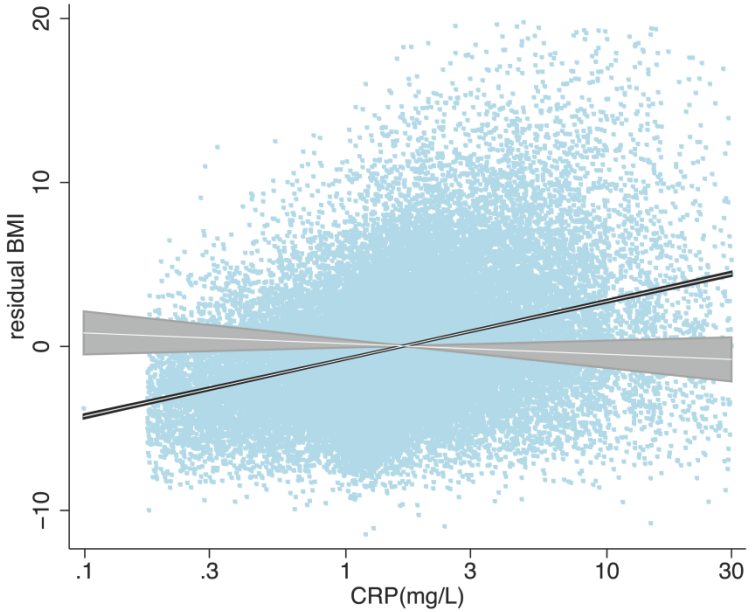
- C-Reactive Protein (CRP) is a biomarker of inflammation
- It is associated with BMI, metabolic syndrome, CHD and a number of other diseases
- It is unclear whether these observational relationships are causal or due to confounding or reverse causality
- This question is important from the perspective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

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



	Effect estimates				
Outcome / explanatory variable	**Observational**	**Instrumental variable**	P_{IV}	P_{diff}	F_{first}
CRP/BMI	1.075 (1.073, 1.077)	1.06 (1.02, 1.11)	0.002	0.6	50.2





Limitations to Mendelian Randomization

1- Population stratification

2- Canalisation (“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

- Weak instruments:
  - Genetic variants that are weak proxies for the exposure
  - Results in biased causal estimates from MR

- Different impact of the bias from weak instruments:
  - **Single Sample MR:** to the confounded estimate
  - **Two-Sample MR:** to the null

Using Multiple Genetic Variants as Instruments

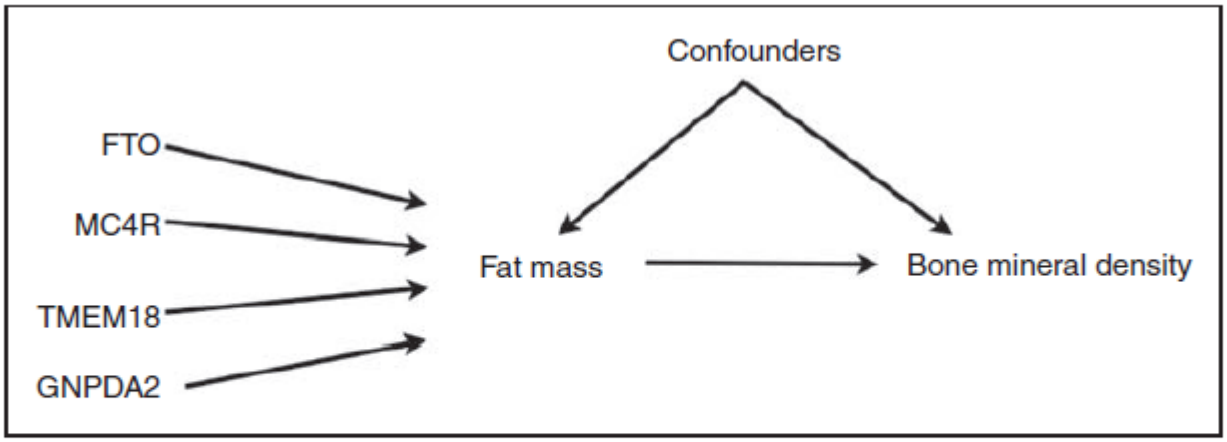


Figure 1. DAG for a Mendelian randomisation analysis using four genetic variants as instrumental variables for the effect of fat mass on bone mineral density.

Palmer et al (2011) Stat Method Res

- Allelic scores
- Testing multiple variants individually
- Meta-analyse individual SNPs

http://cnsgenomics.com/shiny/mRnd/

http://cnsgenomics.com/shiny/mRnd/ mRnd: Power calculations f...

File Edit View Favorites Tools Help

mRnd: Power calculations for Mendelian Randomization

Input

Calculate:

- (•) Power
- ( ) Sample size

Provide:

Sample size

1000

α

0.05

Type-I error rate

β_{yz}

0

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

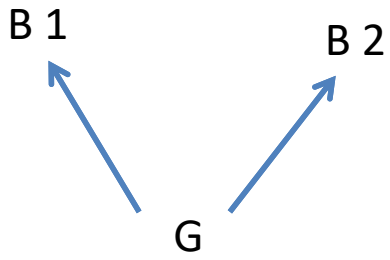
Power	0.05	
NCP	0.00	Non-Centrality-Parameter

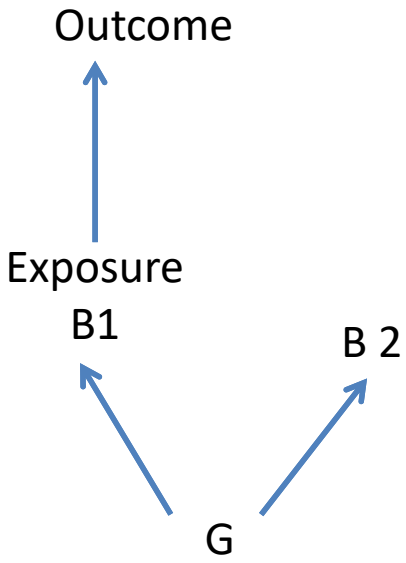
Power or sample size calculations for the regression association of a genetic instrument Z (e.g. a BMI SNP), with a continuous outcome variable Y (blood pressure).

EN 2:26 PM 6/02/2017

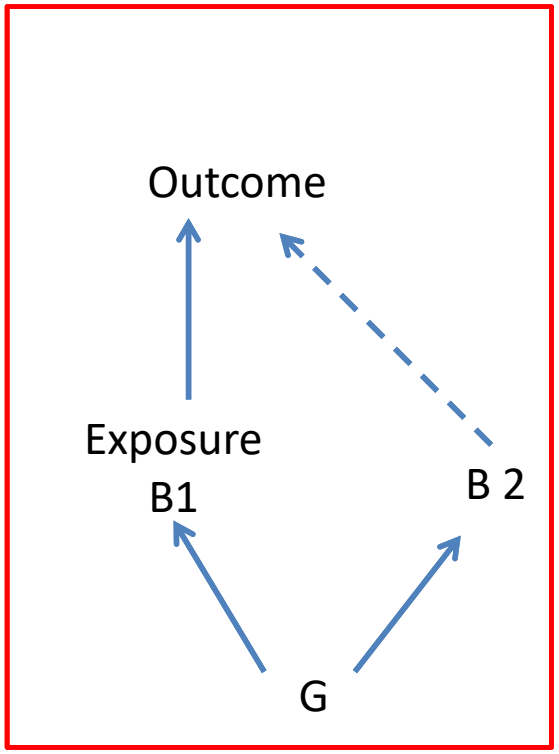
Pleiotropy

- Genetic variant influences more than one trait
- Pleiotropy only violates MR's assumptions if it involves a pathway outside that of the exposure and is a pathway that <u>affects your outcome</u>





Violation



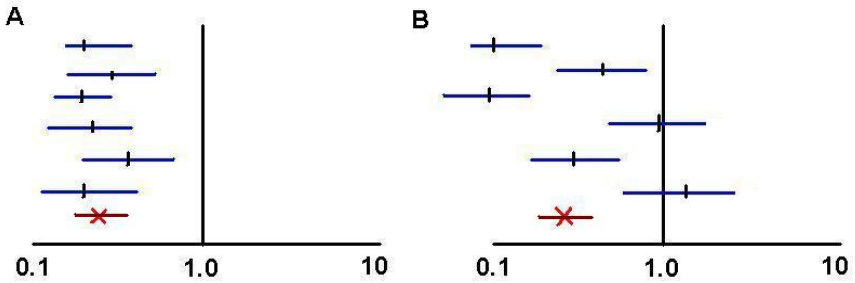
Tests of Heterogeneity to Identify Pleiotropy

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

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



n=6 instruments
Expect Q = 5 if there is no heterogeneity
Q is chi-square distributed with n-1 degrees of freedom

MR Egger regression

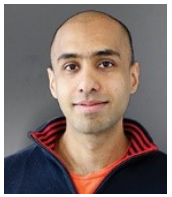
MR Weighted Median

MR Modal Estimator

MR Base

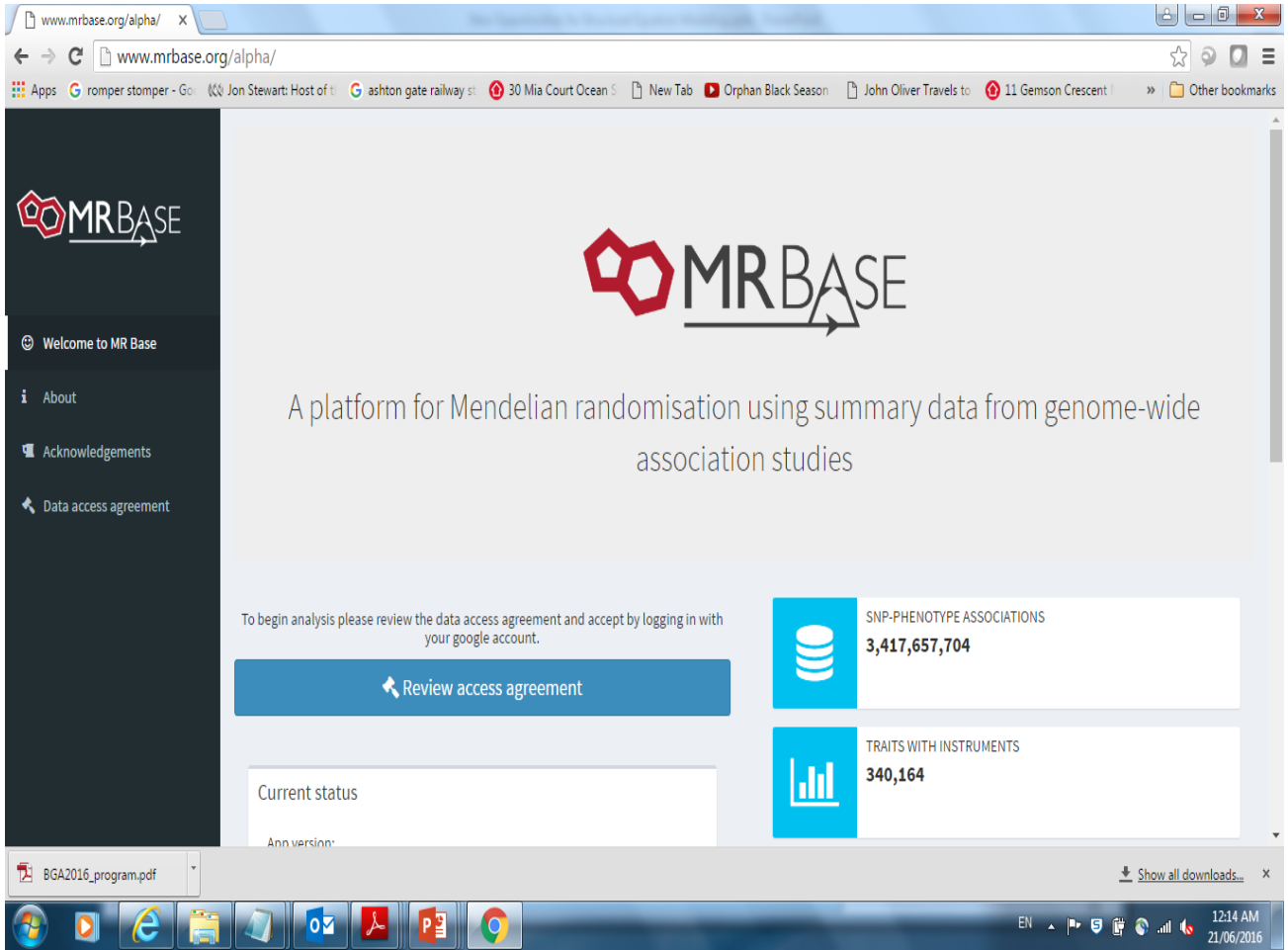
http://www.mrbase.org/

Jie “Chris” Zheng



Gib Hemani

Phil Haycock



MR BASE

- Welcome to MR Base
- About
- Acknowledgements
- Data access agreement

Logged in as
David Evans
epxde@bristol.ac.uk

- **Perform MR analysis**
  - **Choose exposures**
  - Choose outcomes
  - Run MR
- Quick SNP lookup

Choosing instruments for the exposure

To use two sample MR to estimate the causal effect of an exposure on an outcome, the first step is to identify SNPs that are robustly associated with the exposure. These summary statistics for these SNPs can be taken from a sample from which there is no data on the outcome.

Please provide instruments by choosing from one of the data sources below, or by uploading your own data. You can choose multiple exposures to be analysed, and multiple instruments per exposure.

Choose instruments

Select exposure source

- (•) Manual file upload
- ( ) NHGRI-EBI GWAS catalog
- ( ) MR Base GWAS catalog
- ( ) Gene expression QTLs
- ( ) Protein level QTLs
- ( ) Metabolite level QTLs
- ( ) Methylation level QTLs

Manual file upload

The file must be a plain text file.

To do simple SNP look ups it must have at least one column with the header **SNP** .

To do an MR analysis it must have the following column headers:

- **SNP** - rs IDs of the instruments for the exposure
- **beta** - effect sizes for each SNP
- **se** - standard errors
- **effect_allele** - Effect allele

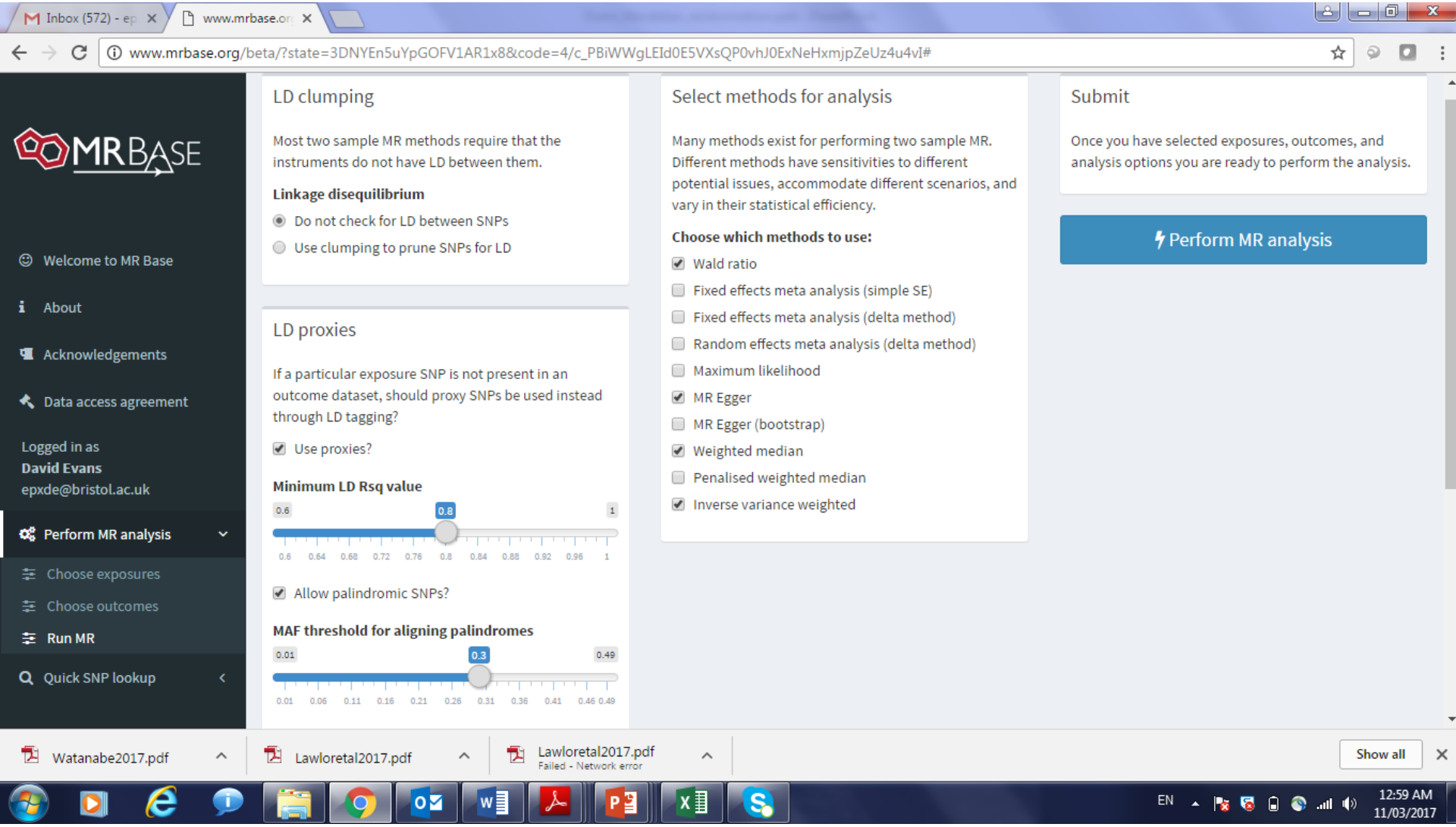
It's useful to have these columns too:

- **other_allele** - Other allele
- **eaf** - Effect allele frequency

You can see an example file here: telomere_length.txt

Upload plain text file *Preview of uploaded table*

Browse... No file s...


Inbox (572) - ep
www.mrbase.org
www.mrbase.org/beta/?state=3DNYEn5uYpGOFV1AR1x8&code=4/c_PBiWWgLEId0E5VXsQP0vhJ0ExNeHxmjpZeUz4u4vI#
MR BASE
Welcome to MR Base
About
Acknowledgements
Data access agreement
Logged in as
David Evans
epxde@bristol.ac.uk
Perform MR analysis
Choose exposures
Choose outcomes
Run MR
Quick SNP lookup
LD clumping
Most two sample MR methods require that the instruments do not have LD between them.
Linkage disequilibrium
Do not check for LD between SNPs
Use clumping to prune SNPs for LD
LD proxies
If a particular exposure SNP is not present in an outcome dataset, should proxy SNPs be used instead through LD tagging?
Use proxies?
Minimum LD Rsq value
0.6 0.8 1
0.6 0.64 0.68 0.72 0.76 0.8 0.84 0.88 0.92 0.96 1
Allow palindromic SNPs?
MAF threshold for aligning palindromes
0.01 0.3 0.49
0.01 0.06 0.11 0.16 0.21 0.26 0.31 0.36 0.41 0.46 0.49
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
Fixed effects meta analysis (simple SE)
Fixed effects meta analysis (delta method)
Random effects meta analysis (delta method)
Maximum likelihood
MR Egger
MR Egger (bootstrap)
Weighted median
Penalised weighted median
Inverse variance weighted
Submit
Once you have selected exposures, outcomes, and analysis options you are ready to perform the analysis.
Perform MR analysis
Watanabe2017.pdf
Lawloretal2017.pdf
Lawloretal2017.pdf
Failed - Network error
Show all
EN
12:59 AM
11/03/2017


Inbox (572) - epxde@bris
www.mrbase.org/beta/?s
www.mrbase.org/beta/?state=3DNYEn5uYpGOFV1AR1x8&code=4/c_PBiWWgLEId0E5VXsQP0vhJ0ExNeHxmjpZeUz4u4vI#
Run MR
MR Results
Quick SNP lookup
5e-08
Perform clumping
Display columns
ID
Trait
Note
First author
Consortium
Number of cases
Number of controls
Sample size
Number of variants
Year
PubmedID
Access
Category
Population
Priority
Sd
Sex
Subcategory
Unit
Search: ldl
ID Trait Note First author Consortium Number of cases Number of controls Sample size Number of variants Year PubmedID Access Category Po
300 300 LDL cholesterol Willer CJ GLGC 173082 2437752 2013 24097068 public Risk factor
781 781 LDL cholesterol Metabo-chip Willer CJ GLGC 83198 120251 2013 24097068 public Risk factor
880 880 Total cholesterol in large LDL L.LDL.C Kettunen 21552 11871461 2016 27005778 public Metabolites
881 881 Cholesterol esters in large VLDL L.LDL.CE Kettunen 19273 11820655 2016 27005778 public Metabolites
Watanabe2017.pdf
Lawloretal2017.pdf
Lawloretal2017.pdf
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Show all
EN
1:10 AM
11/03/2017

Display columns

- [ ] ID
- [x] Trait
- [x] Note
- [x] First author
- [x] Consortium
- [x] Number of cases
- [x] Number of controls
- [x] Sample size
- [x] Number of variants
- [x] Year
- [ ] PubmedID
- [ ] Access
- [ ] Category
- [ ] Population
- [ ] Priority
- [ ] Sd
- [ ] Sex
- [x] Subcategory
- [ ] Unit

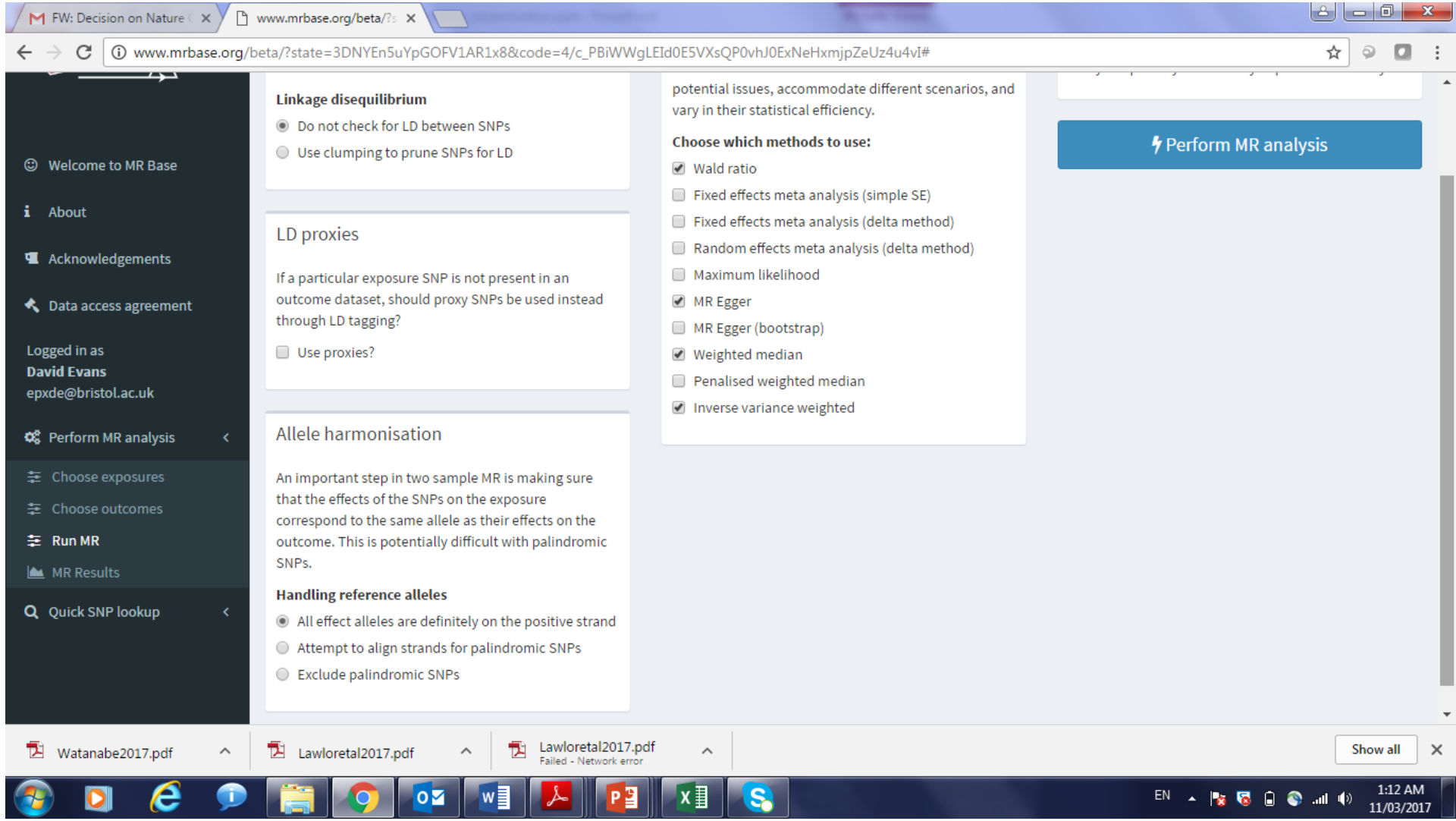
Show 10 entries

Search: coro

	Trait	Note	First author	Consortium	Number of cases	Number of controls	Sample size	Number of variants	Year	Subcategory
6	Coronary heart disease		Peden	C4D	15420	15062	30482	540233	2011	Cardiovascular
7	**Coronary heart disease**		**Nikpay**	**CARDIoGRAMplusC4D**	**60801**	**123504**	**184305**	**9455779**	**2015**	**Cardiovascular**
8	Coronary heart disease		Schunkert H	CARDIoGRAM	22233	64762	86995	2420361	2011	Cardiovascular
9	Coronary heart disease		Deloukas	CARDIoGRAMplusC4D	63746	130681	194427	79129	2013	Cardiovascular
	All	All	All	All	All	All	All	All	All	All

Showing 1 to 4 of 4 entries (filtered from 1,033 total entries)

Previous 1 Next


FW: Decision on Nature
www.mrbase.org/beta/?s
www.mrbase.org/beta/?state=3DNYEn5uYpGOFV1AR1x8&code=4/c_PBiWWgLEId0E5VXsQP0vhJ0ExNeHxmjpZeUz4u4vI#
Welcome to MR Base
About
Acknowledgements
Data access agreement
Logged in as
David Evans
epxde@bristol.ac.uk
Perform MR analysis
Choose exposures
Choose outcomes
Run MR
MR Results
Quick SNP lookup
Linkage disequilibrium
Do not check for LD between SNPs
Use clumping to prune SNPs for LD
LD proxies
If a particular exposure SNP is not present in an outcome dataset, should proxy SNPs be used instead through LD tagging?
Use proxies?
Allele harmonisation
An important step in two sample MR is making sure that the effects of the SNPs on the exposure correspond to the same allele as their effects on the outcome. This is potentially difficult with palindromic SNPs.
Handling reference alleles
All effect alleles are definitely on the positive strand
Attempt to align strands for palindromic SNPs
Exclude palindromic SNPs
potential issues, accommodate different scenarios, and vary in their statistical efficiency.
Choose which methods to use:
Wald ratio
Fixed effects meta analysis (simple SE)
Fixed effects meta analysis (delta method)
Random effects meta analysis (delta method)
Maximum likelihood
MR Egger
MR Egger (bootstrap)
Weighted median
Penalised weighted median
Inverse variance weighted
Perform MR analysis
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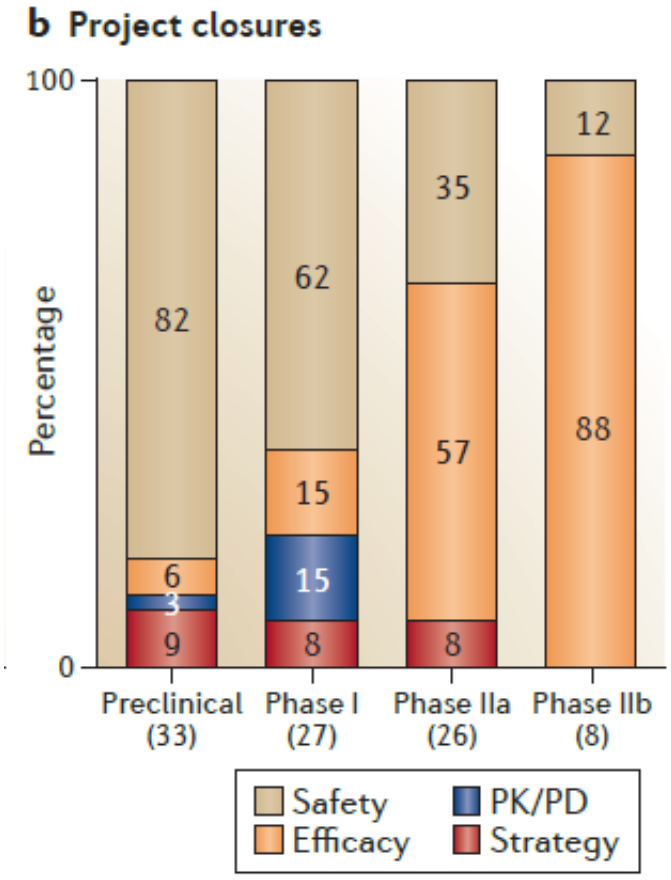
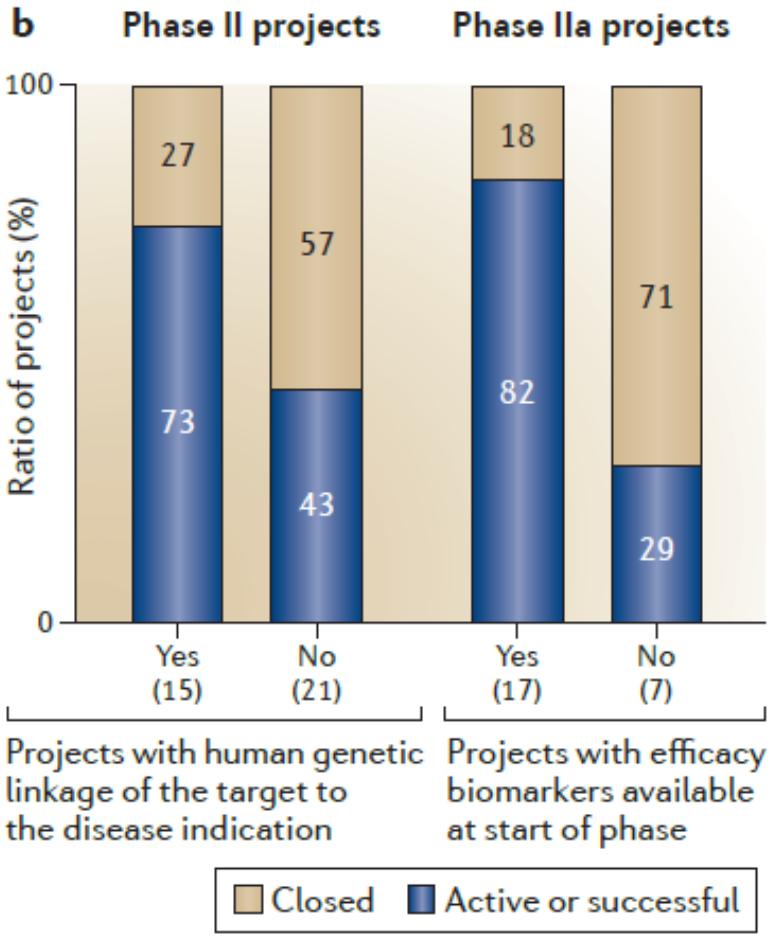
11/03/2017

Mendelian Randomization and Drug Targets

Thanks Sek Kathiresan

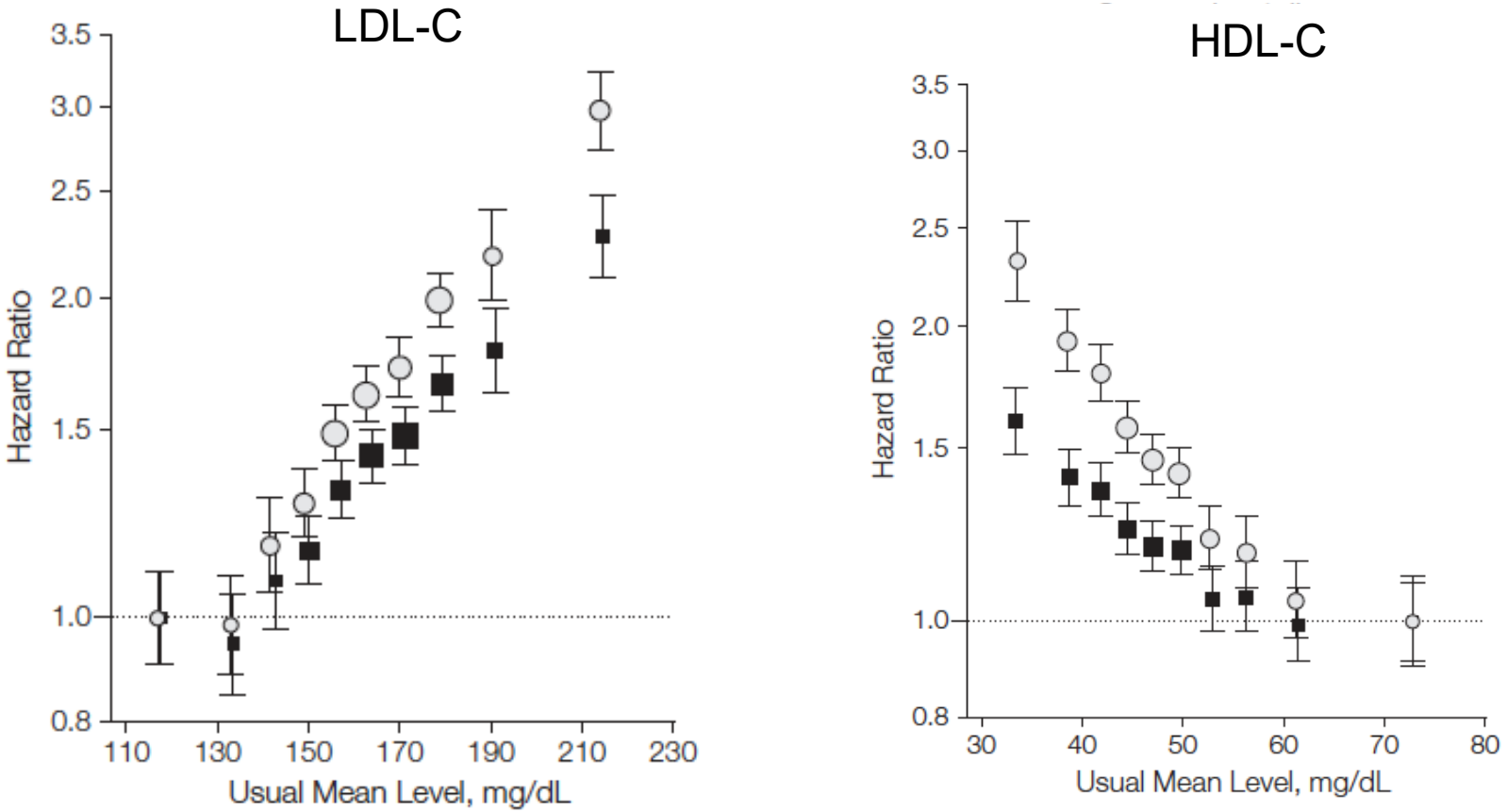
Late Stage Failure in Drug Trials





Cook et al. (2014) *Nat Rev Drug Disc*

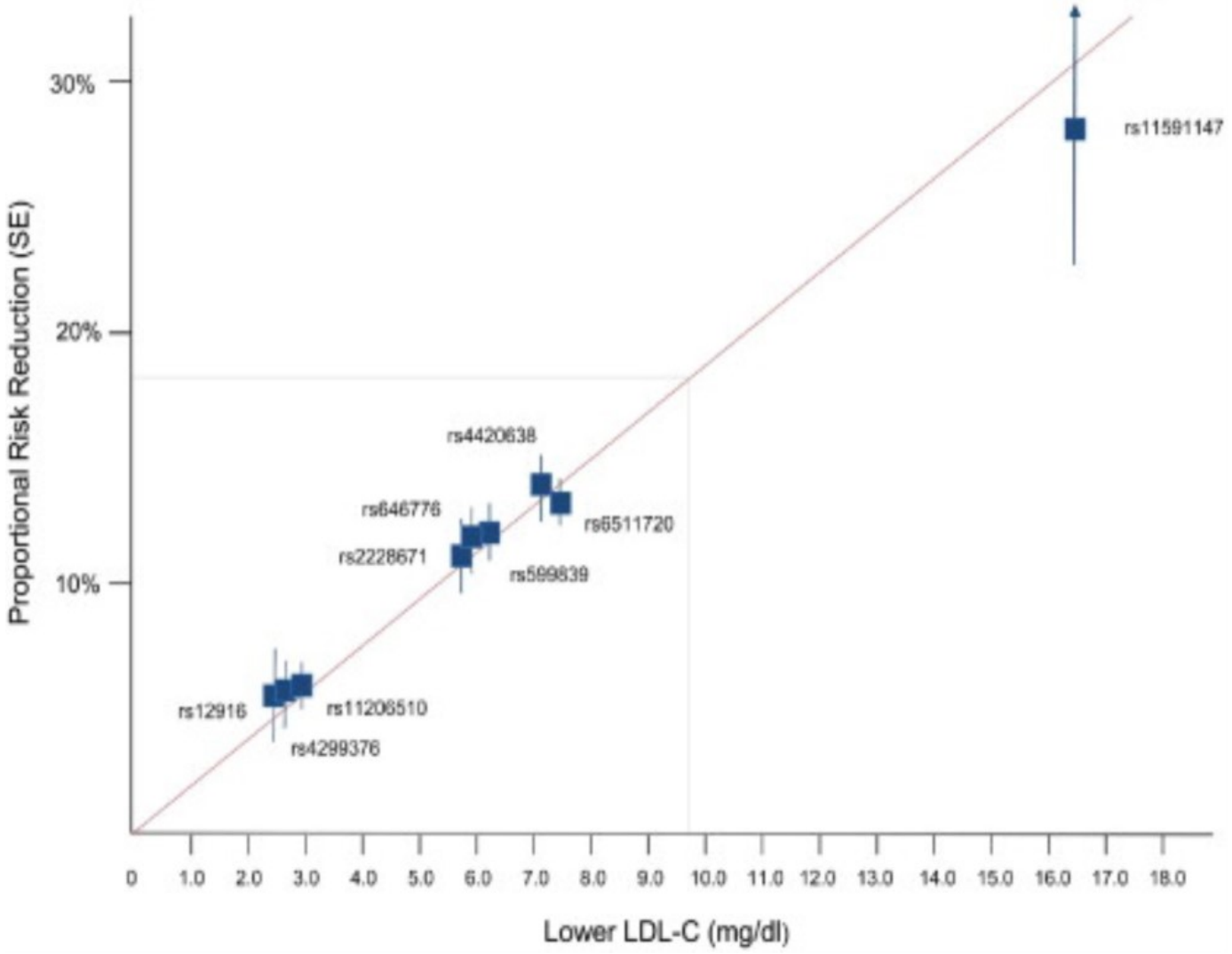
Association of LDL-C, HDL-C, and risk for coronary heart disease (CHD)



302K participants in 68 prospective studies

Emerging Risk Factors Collaboration, *JAMA* 2009

LDL and CHD Risk



Ference et al, JACC 2012

HDL: endothelial lipase Asn396Ser

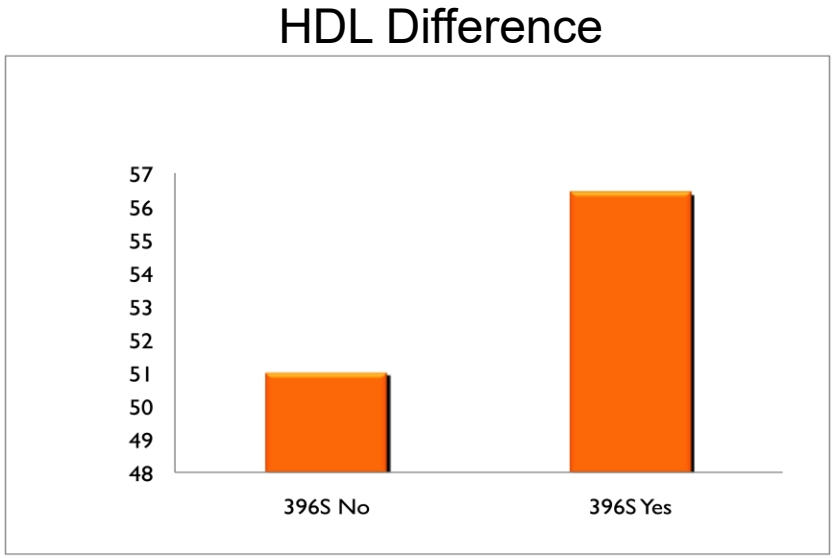
Loss-of-function variants in endothelial lipase are a cause of elevated HDL cholesterol in humans

Andrew C. Edmondson,[1] Robert J. Brown,[1] Sekar Kathiresan,[2,3] L. Adrienne Cupples,[4] Serkalem Demissie,[4] Alisa Knodle Manning,[4] Majken K. Jensen,[5] Eric B. Rimm,[5,6] Jian Wang,[7] Amrith Rodrigues,[1] Vaneeta Bamba,[1] Sumeet A. Khetarpal,[1] Megan L. Wolfe,[1] Stephanie DerOhannessian,[1] Mingyao Li,[8] Muredach P. Reilly,[1,9] Jens Aberle,[10] David Evans,[10] Robert A. Hegele,[7] and Daniel J. Rader[1,9]

- 2.6% of population carry Serine allele
- higher HDL-C
- No effect on other lipid fractions
- No effect on other MI risk factors

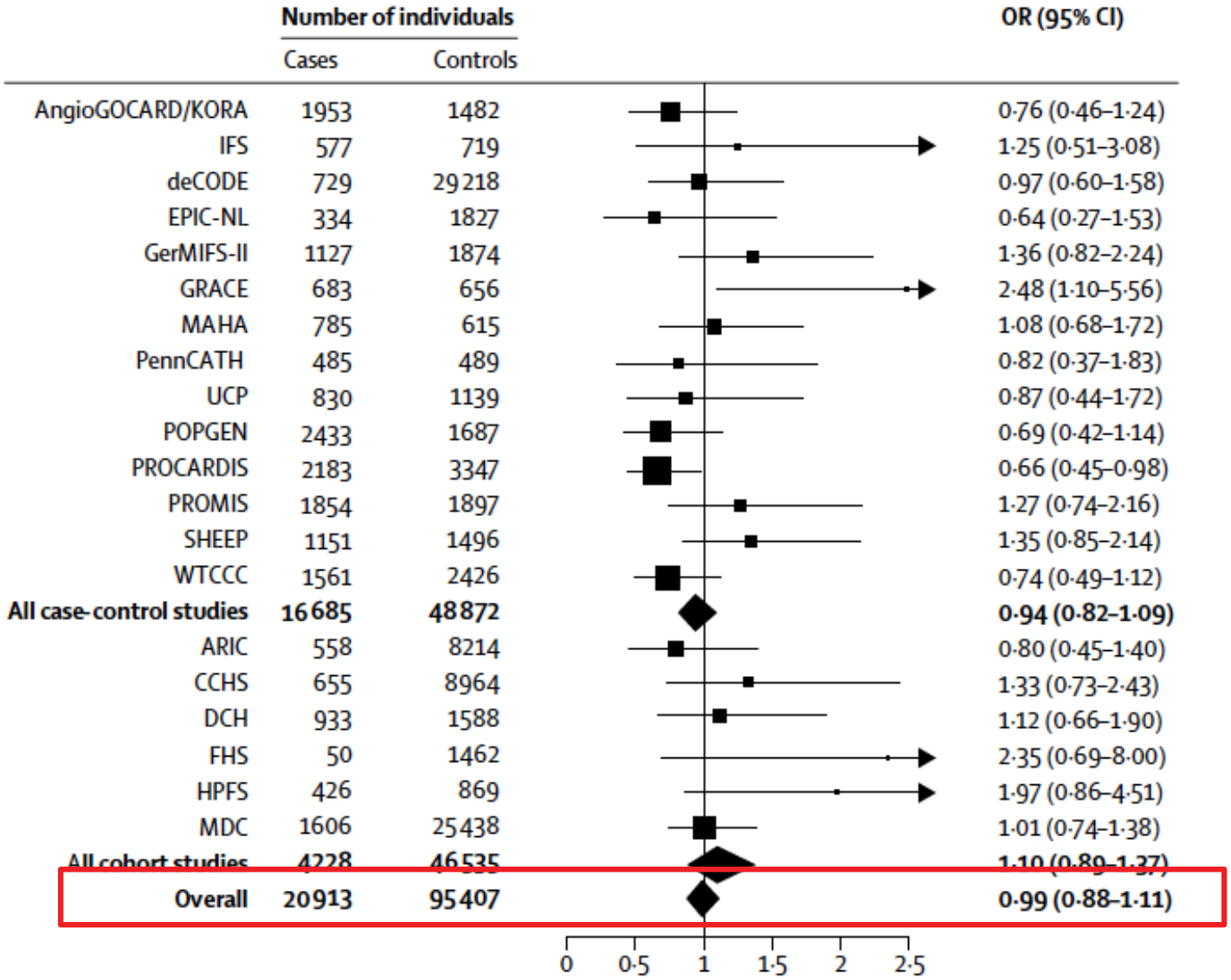
Edmondson, *J Clin Invest* 2009

LIPG N396S and plasma HDL-C



396S carriers have
5.5 mg/dl higher HDL-C
$P<10^{-8}$

After testing in 116,320 people, summary OR for *LIPG* Asn396Ser is 0.99



Individuals who carry the HDL-boosting variant
have the same risk for heart attack
as those who do not carry the variant

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 NOVEMBER 22, 2007 VOL. 357 NO. 21

Effects of Torcetrapib in Patients at High Risk for Coronary Events

Philip J. Barter, M.D., Ph.D., Mark Caulfield, M.D., M.B., B.S., Mats Eriksson, M.D., Ph.D., Scott M. Grundy, M.D., Ph.D., John J.P. Kastelein, M.D., Ph.D., Michel Komajda, M.D., Jose Lopez-Sendon, M.D., Ph.D., Lori Mosca, M.D., M.P.H., Ph.D., Jean-Claude Tardif, M.D., David D. Waters, M.D., Charles L. Shear, Dr.P.H., James H. Revkin, M.D., Kevin A. Buhr, Ph.D., Marian R. Fisher, Ph.D., Alan R. Tall, M.B., B.S., and Bryan Brewer, M.D., Ph.D., for the ILLUMINATE Investigators*

RESULTS

protein cholesterol, as compared with baseline ($P<0.001$ for both comparisons), in addition to an increase of 5.4 mm Hg in systolic blood pressure, a decrease in serum potassium, and increases in serum sodium, bicarbonate, and aldosterone ($P<0.001$

showed an increased risk of death in patients treated with torcetrapib whose reduction in potassium or increase in bicarbonate was greater than the median change.

ORIGINAL ARTICLE

Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome

Gregory G. Schwartz, M.D., Ph.D., Anders G. Olsson, M.D., Ph.D., Markus Abt, Ph.D., Christie M. Ballantyne, M.D., Philip J. Barter, M.D., Ph.D., Jochen Brumm, Ph.D., Bernard R. Chaitman, M.D., Ingar M. Holme, Ph.D., David Kallend, M.B., B.S., Lawrence A. Leiter, M.D., Eran Leitersdorf, M.D., John J.V. McMurray, M.D., Hardi Mundl, M.D., Stephen J. Nicholls, M.B., B.S., Ph.D., Prediman K. Shah, M.D., Jean-Claude Tardif, M.D., and R. Scott Wright, M.D., for the dal-OUTCOMES Investigators*

RESULTS

At the time of randomization, the mean HDL cholesterol level was 42 mg per deciliter (1.1 mmol per liter), and the mean low-density lipoprotein (LDL) cholesterol

interim analysis that included 1135 primary end-point events (71% of the projected total number), the independent data and safety monitoring board recommended

per liter higher and the mean systolic blood pressure was 0.6 mm Hg higher with dalcetrapib as compared with placebo (P<0.001 for both comparisons).

OPINION

HDL—is it too big to fail?

Dominic S. Ng, Norman C. W. Wong and Robert A. Hegele

Abstract | The HDL hypothesis has suffered damage in the past few years. Clinical trials have shown that raising HDL cholesterol levels does not improve cardiovascular disease (CVD) outcomes. In addition, Mendelian randomization studies have shown that DNA variants that alter HDL cholesterol levels in populations are unrelated to incident CVD events. Balancing this deluge of negative data are substantial basic science data supporting the concept that raising HDL cholesterol levels reduces CVD risk. Also, functionally relevant HDL subfractions might be more important determinants of risk than overall HDL cholesterol levels. But, while wobbly, the HDL hypothesis is still standing, seemingly too big to fail owing to past intellectual, economic and psychological investments in the idea.

Ng, D. S. *et al. Nat. Rev. Endocrinol.* **9**, 308–312 (2013); published online 15 January 2013;
doi:10.1038/nrendo.2012.238

Useful References

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Practical