Using Mendelian randomization to explore causality

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Drug development in the genomics era

More than half of the drugs thought promising in the lab will go on to fail

Ernesto del Aguila, NHGRI

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Drug development in the genomics era

More than half of the drugs thought promising in the lab will go on to fail

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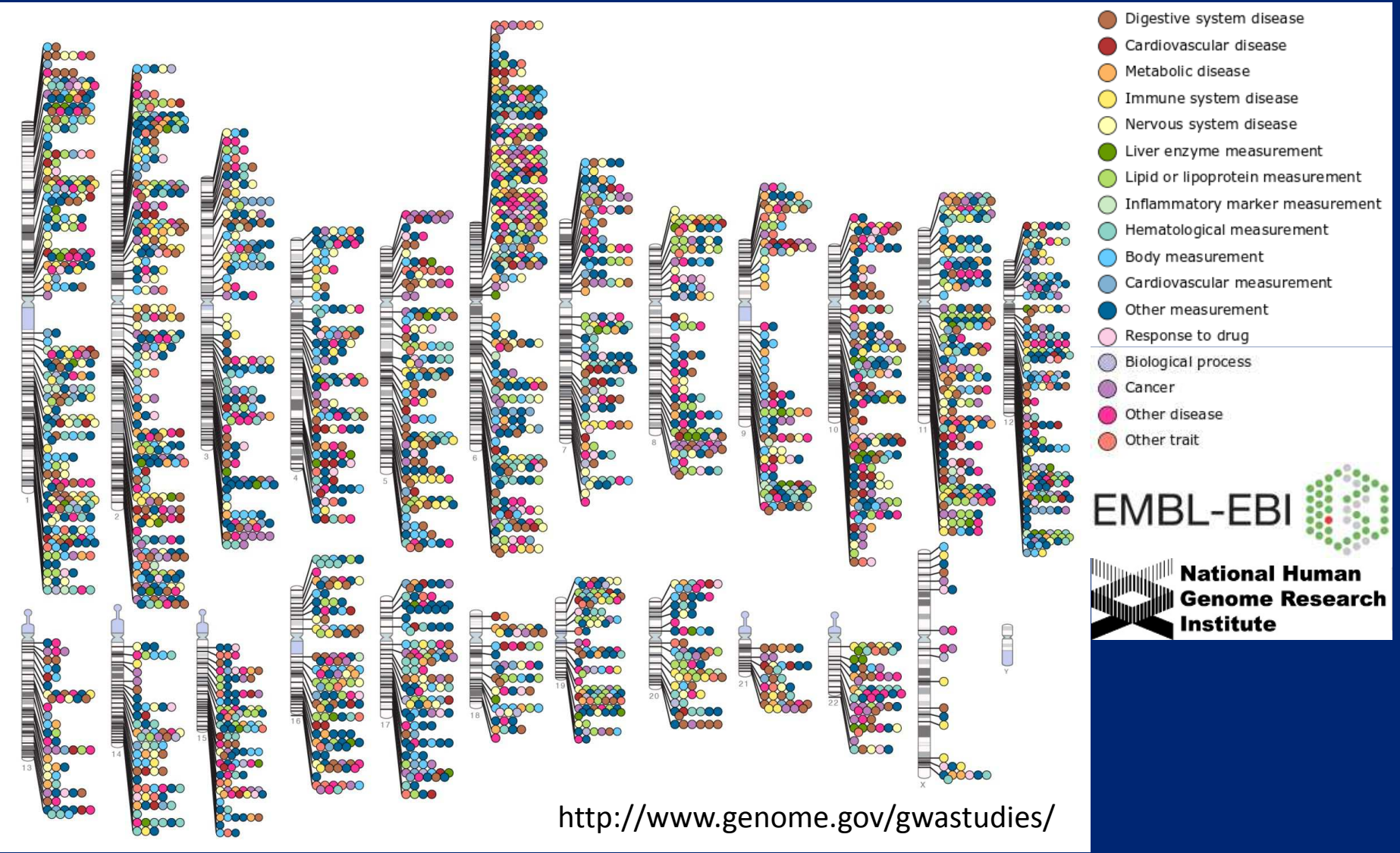
Can we use genomic information to improve this success rate?

Ernesto del Aguila, NHGRI

The wealth of genetic data



GWA studies of cardiovascular traits

Large-scale genome-wide association (GWA) meta-analyses, often involving trans-ethnic studies, offer large-scale information on

- Coronary heart disease (~60k cases)
- Ischaemic stroke (~12k cases)
- Lipid fractions (~200k individuals)
- Blood pressure (~350k individuals)
- Inflammatory markers, etc… (~100k individuals)
- Population biobanks (e.g. ~500k individuals in UKB)

Translational potential of genetic data

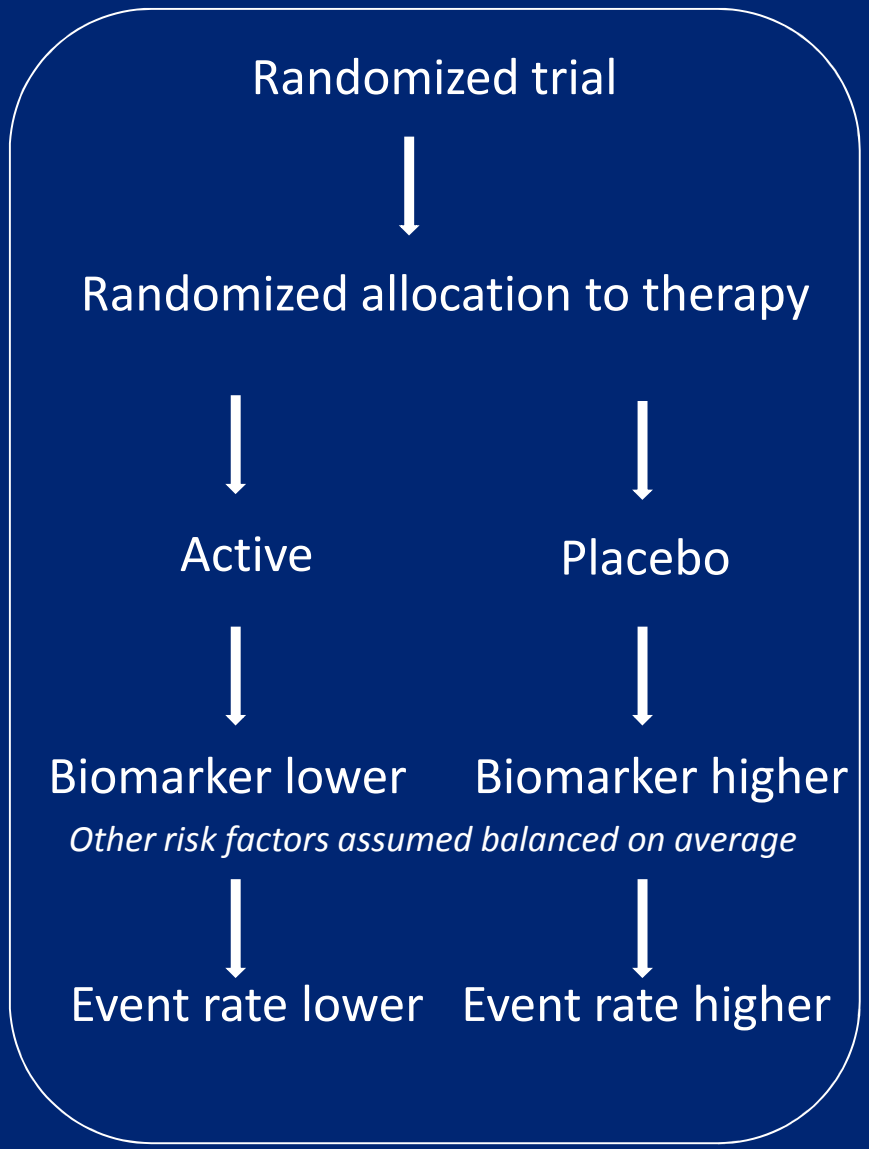
Large-scale genomic studies have the potential to be used to identify new therapeutic targets as well as to:

- Explore the causal effects of potential therapeutic pathways on disease

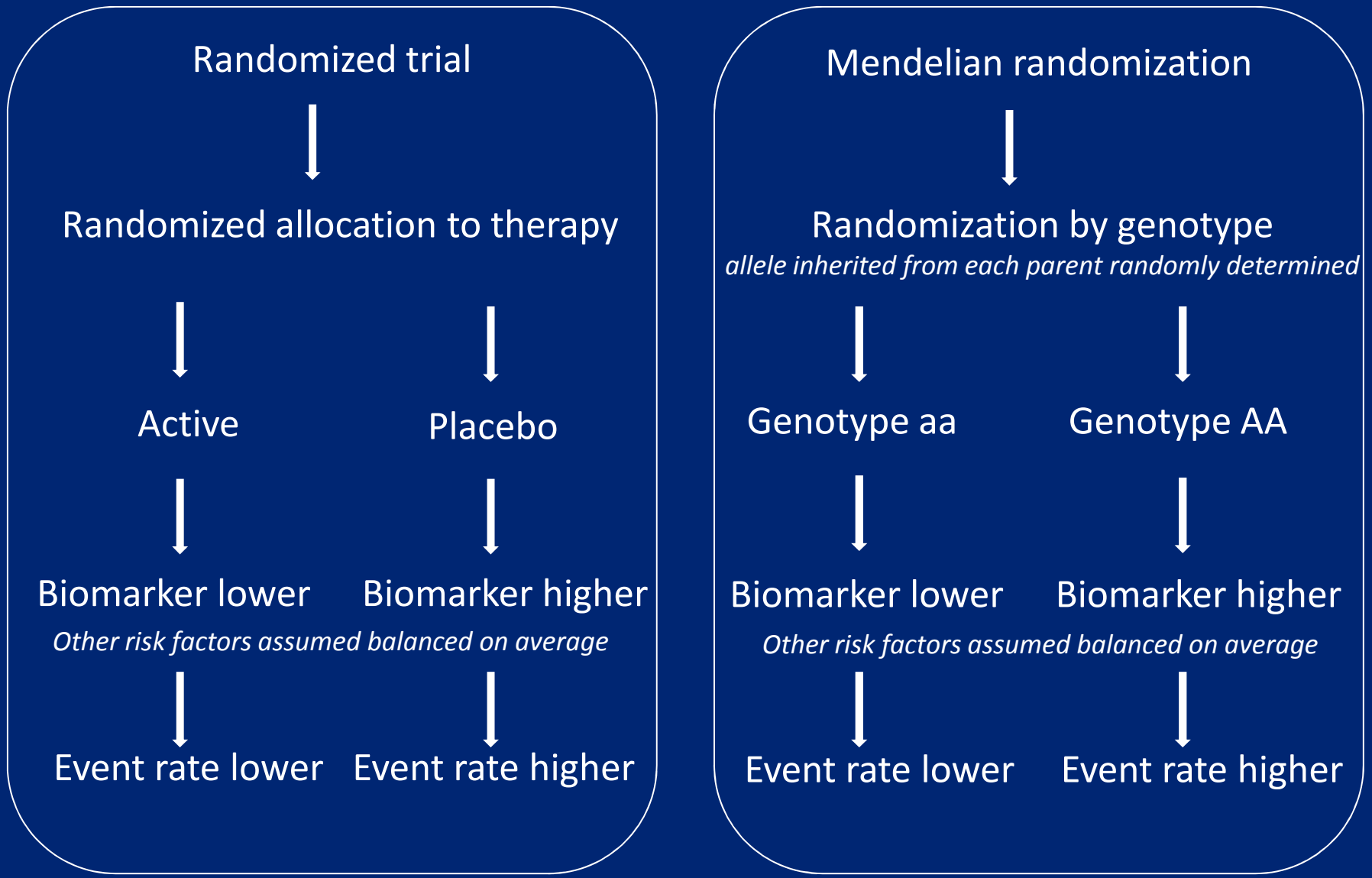
- Explore causality of biomarkers associated with disease risk

by examining the effects of genetic variants (and genetic risk scores) that can mimic the risk factor or drug target of interest.

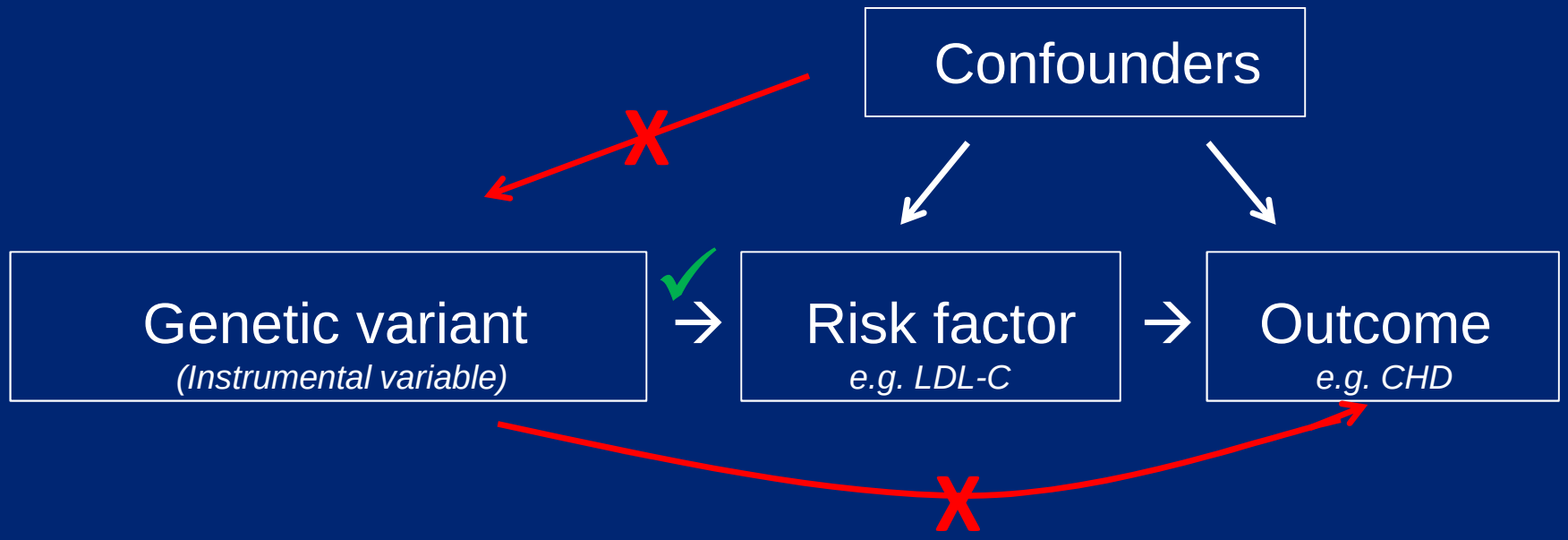
Randomized trials vs Mendelian randomization



Randomized trials vs Mendelian randomization



Genetic variants for Mendelian Randomization

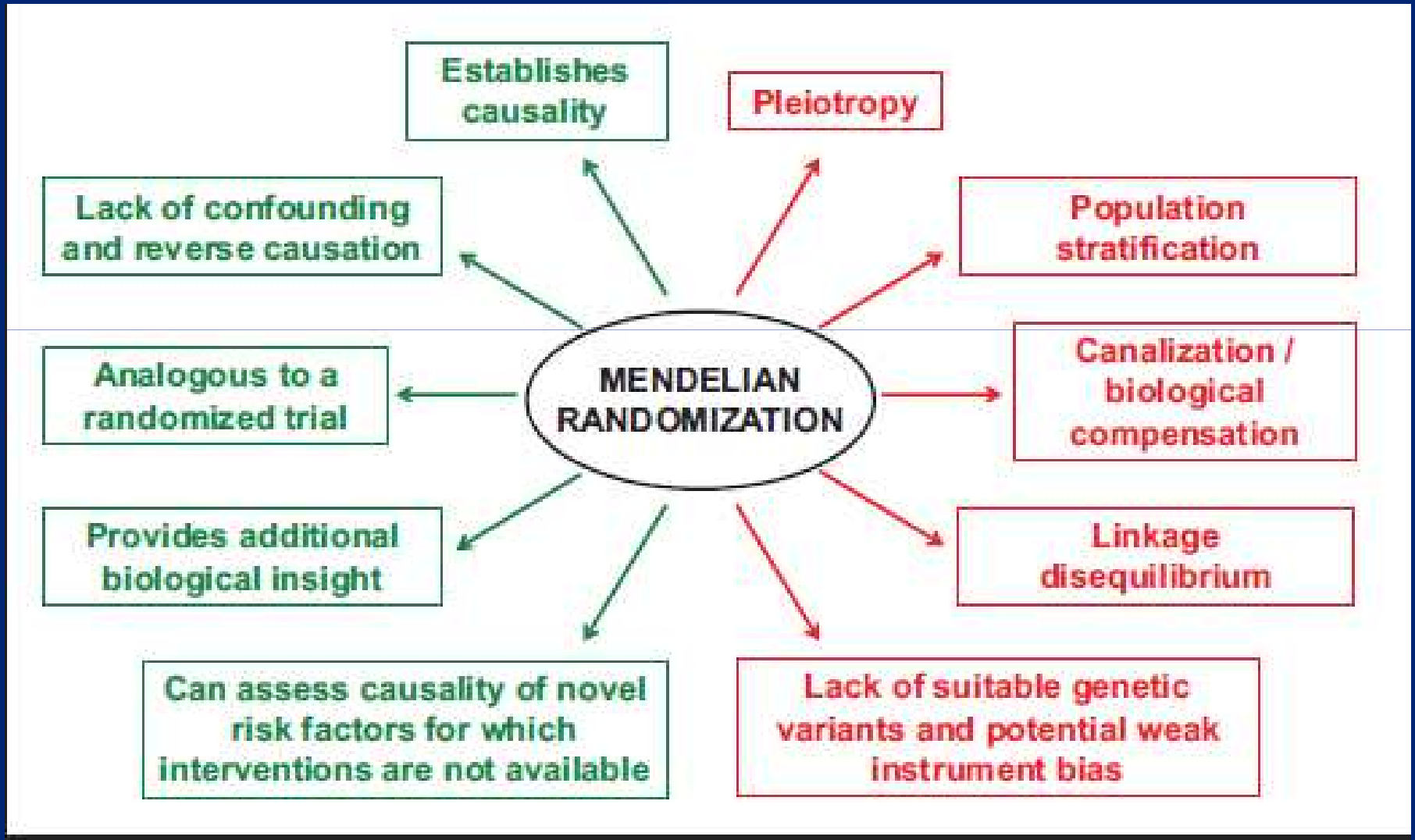


1. The genetic variant should be associated with the risk factor

2. The genetic variant should not associated with a confounder of the risk factor – outcome association

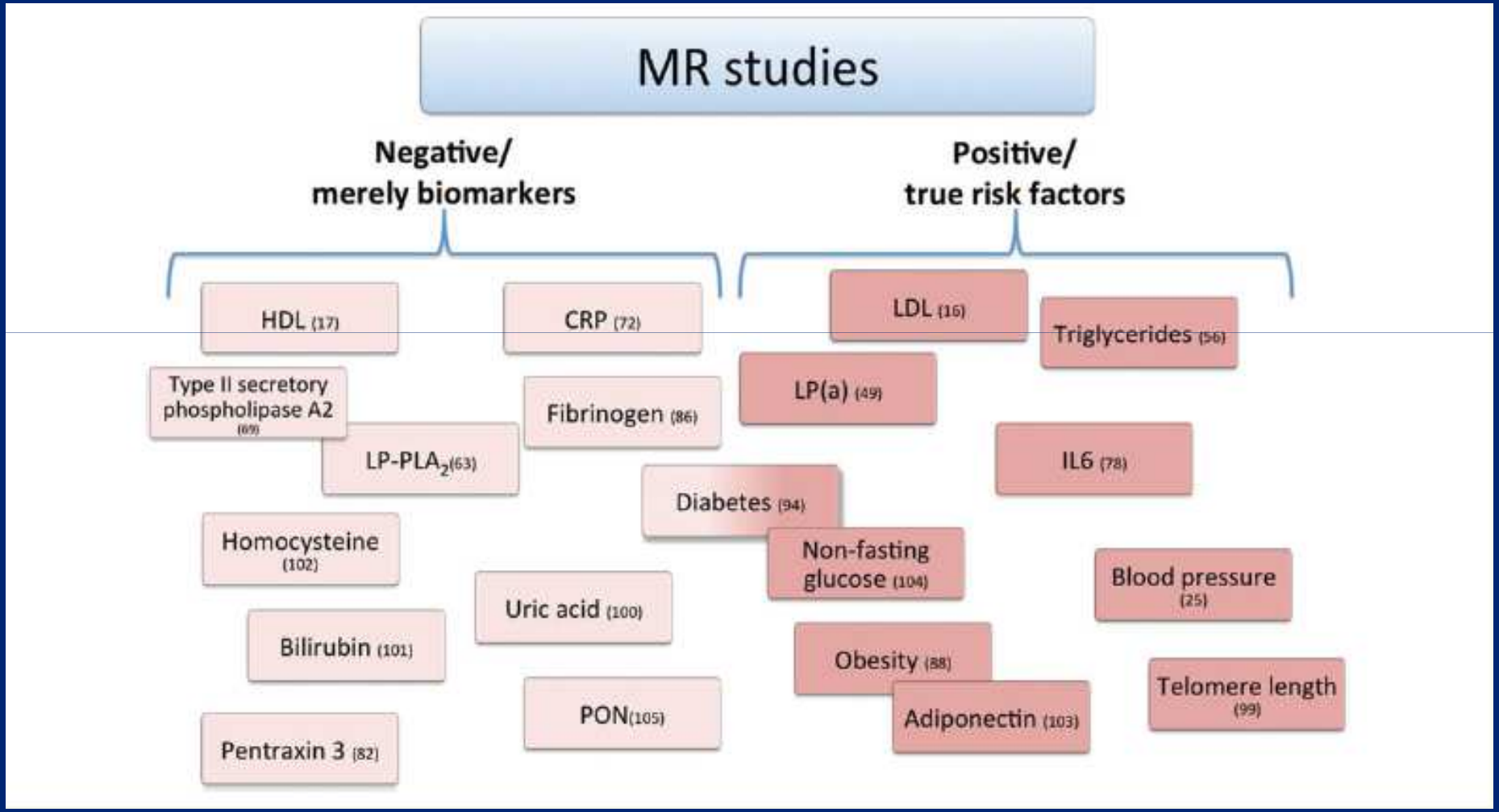
3. There should be no alternative causal pathway from the genetic variant to the outcome, except for via the risk factor

Advantages and limitations



Hopewell et al, Stroke, 2016

MR studies of coronary heart disease



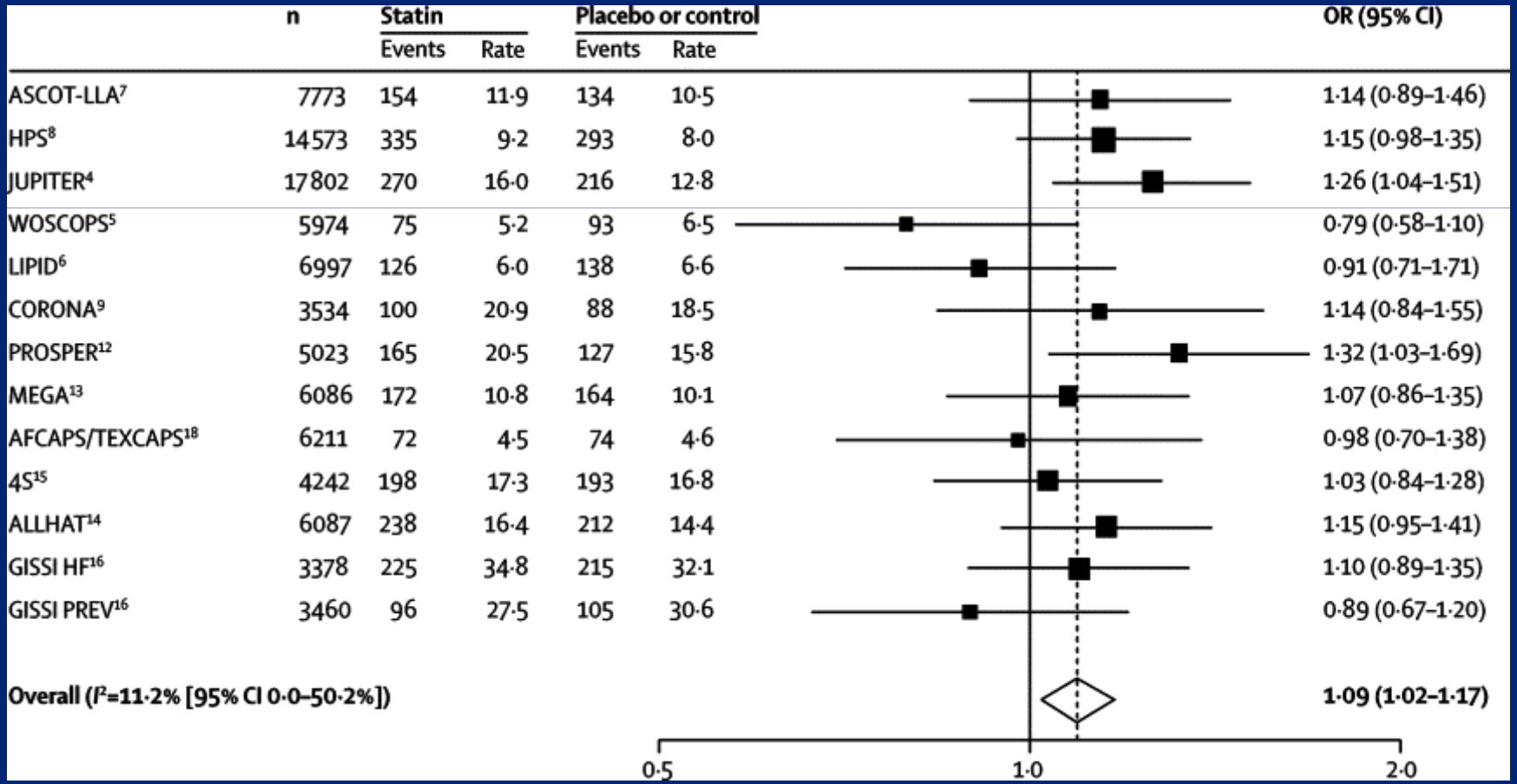
Jansen et al, EHJ, 2014

Can genetic studies anticipate causal on-target adverse effects?

Statins & new-onset diabetes

Statins and diabetes – the trial evidence

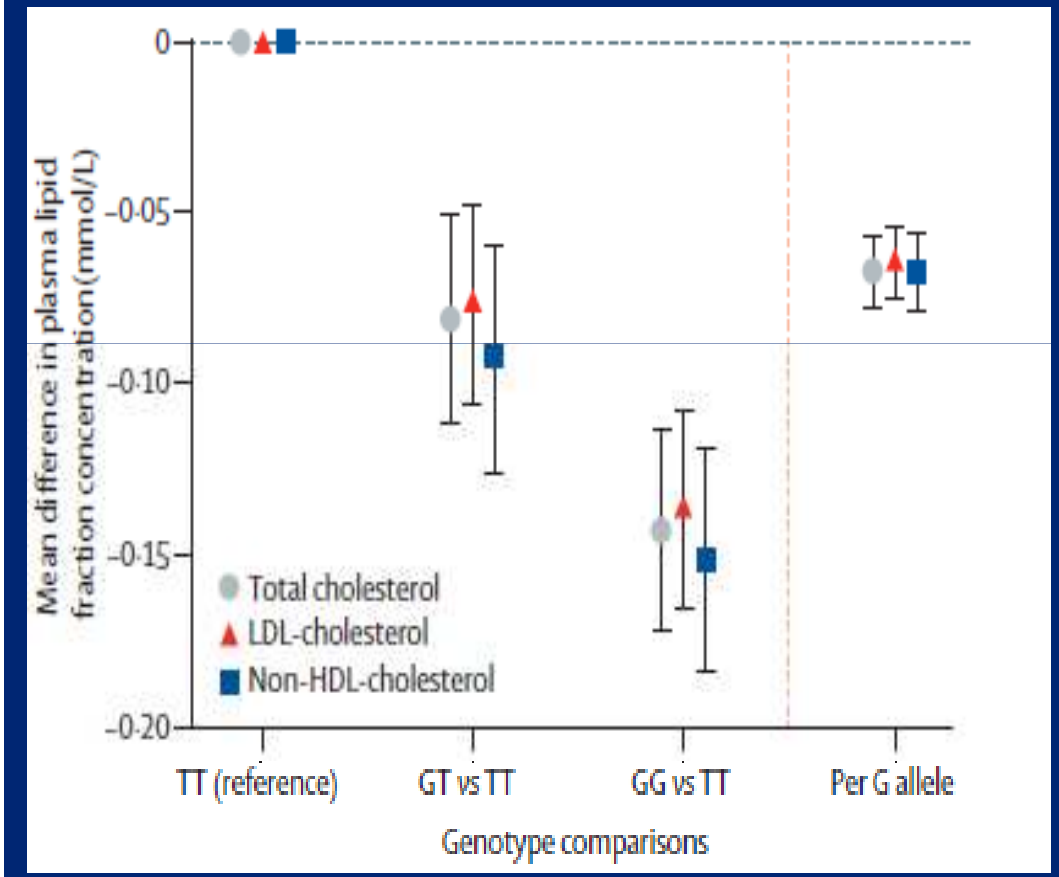
- 91, 140 statin trial participants, of whom 4278 developed diabetes during a mean of 4 years.



	n	Statin		Placebo or control		OR (95% CI)
		Events	Rate	Events	Rate	
ASCOT-LLA[7]	7773	154	11·9	134	10·5	1·14 (0·89–1·46)
HPS[8]	14573	335	9·2	293	8·0	1·15 (0·98–1·35)
JUPITER[4]	17802	270	16·0	216	12·8	1·26 (1·04–1·51)
WOSCOPS[5]	5974	75	5·2	93	6·5	0·79 (0·58–1·10)
LIPID[6]	6997	126	6·0	138	6·6	0·91 (0·71–1·71)
CORONA[9]	3534	100	20·9	88	18·5	1·14 (0·84–1·55)
PROSPER[12]	5023	165	20·5	127	15·8	1·32 (1·03–1·69)
MEGA[13]	6086	172	10·8	164	10·1	1·07 (0·86–1·35)
AFCAPS/TEXCAPS[18]	6211	72	4·5	74	4·6	0·98 (0·70–1·38)
4S[15]	4242	198	17·3	193	16·8	1·03 (0·84–1·28)
ALLHAT[14]	6087	238	16·4	212	14·4	1·15 (0·95–1·41)
GISSI HF[16]	3378	225	34·8	215	32·1	1·10 (0·89–1·35)
GISSI PREV[16]	3460	96	27·5	105	30·6	0·89 (0·67–1·20)
Overall (I^2=11·2% [95% CI 0·0–50·2%])						**1·09 (1·02–1·17)**

Sattar et al. Lancet, 2010

HMGCR genetic variants mimic statin use

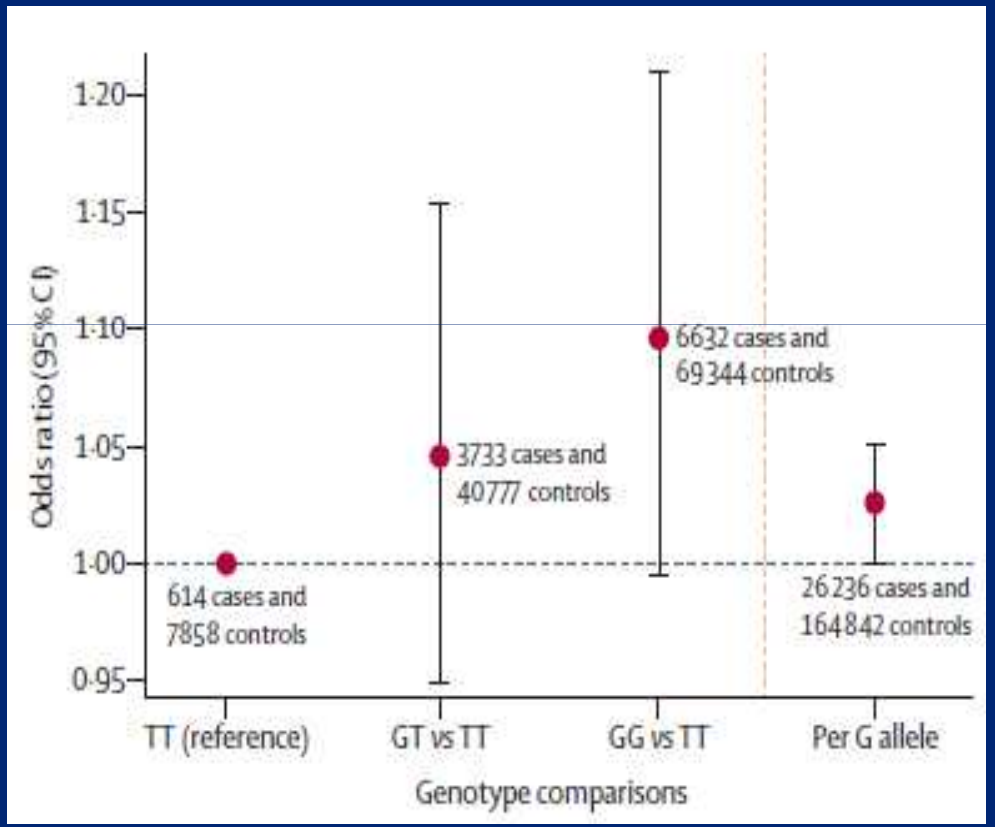


- Statins exert their action by inhibiting HMGCR, leading to LDL-reduction.
- *A HMGCR* genetic variant, used as a proxy for statin use, was associated with lower LDL-C.

Swerdlow, Lancet, 2015

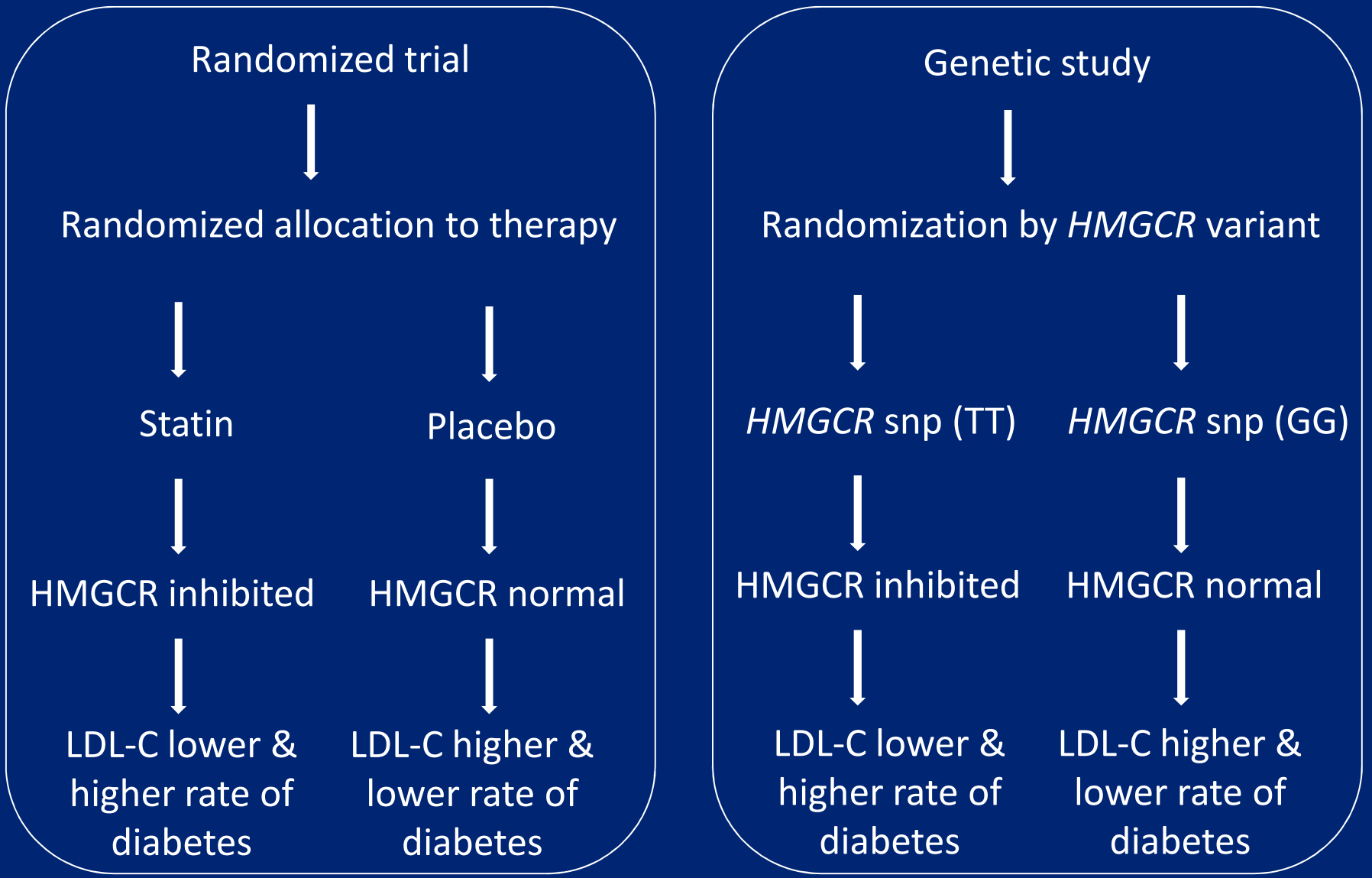
Is *HMGCR* associated with diabetes?

- The *HMGCR* genetic variant associated with lower LDL-C was also associated with higher risk of new onset diabetes.
- Impact on diabetes is an on-target effect of HMGCR inhibition.



Swerdlow, Lancet, 2015

Statins – Trials vs Genetic studies



Implications

- Life-long inhibition of HMGCR is associated with lower LDL-C and higher risk of new onset diabetes. Effect of statins on diabetes at least partially explained by HMGCR inhibition.

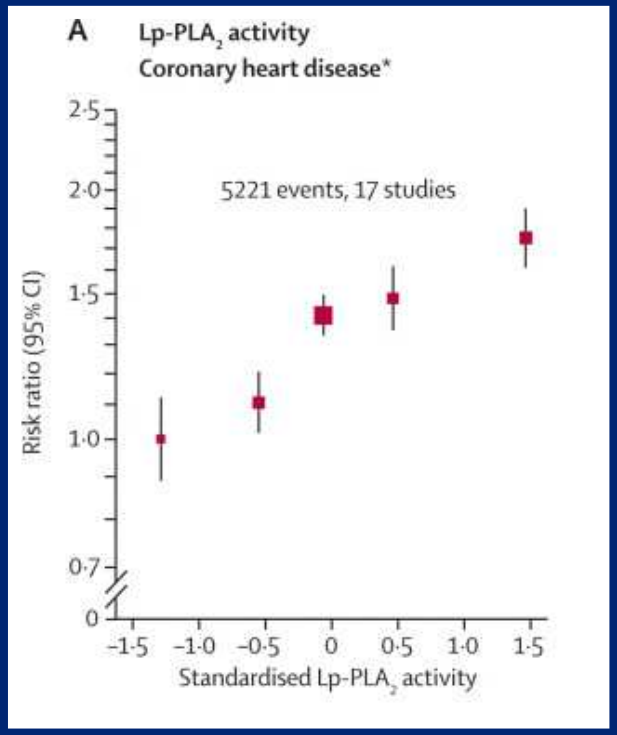
- Small effect on diabetes requires large-scale studies. PheWAS may offer further insight into potential small on-target effects, albeit cannot assess off-target effects.

Can genetic studies identify a poor choice of therapeutic target?

$LpPLA_2$ inhibition & vascular events

Lp-PLA_2 - observational and trial evidence

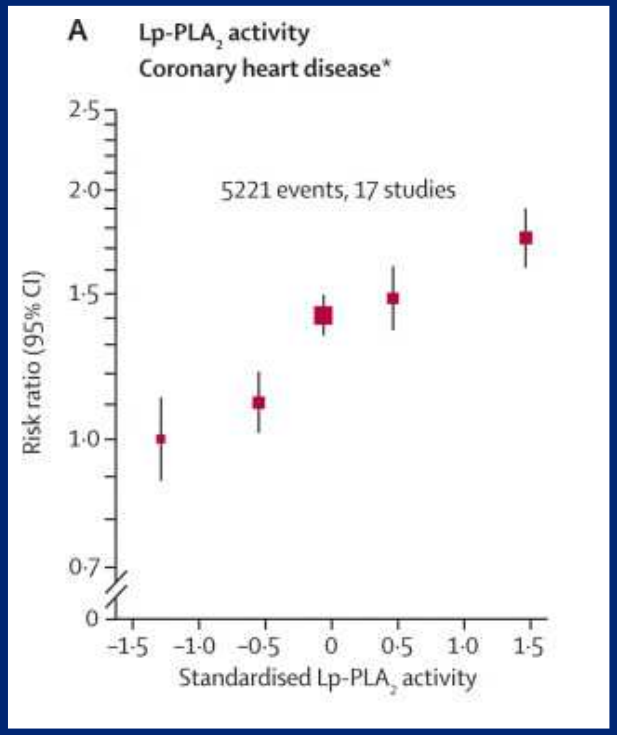
- Lp-PLA_2 produces pro-inflammatory mediators through hydrolysis of oxidised phospholipids carried on LDL in atherosclerotic plaques.
- Increased Lp-PLA_2 activity associated with higher CHD risk.



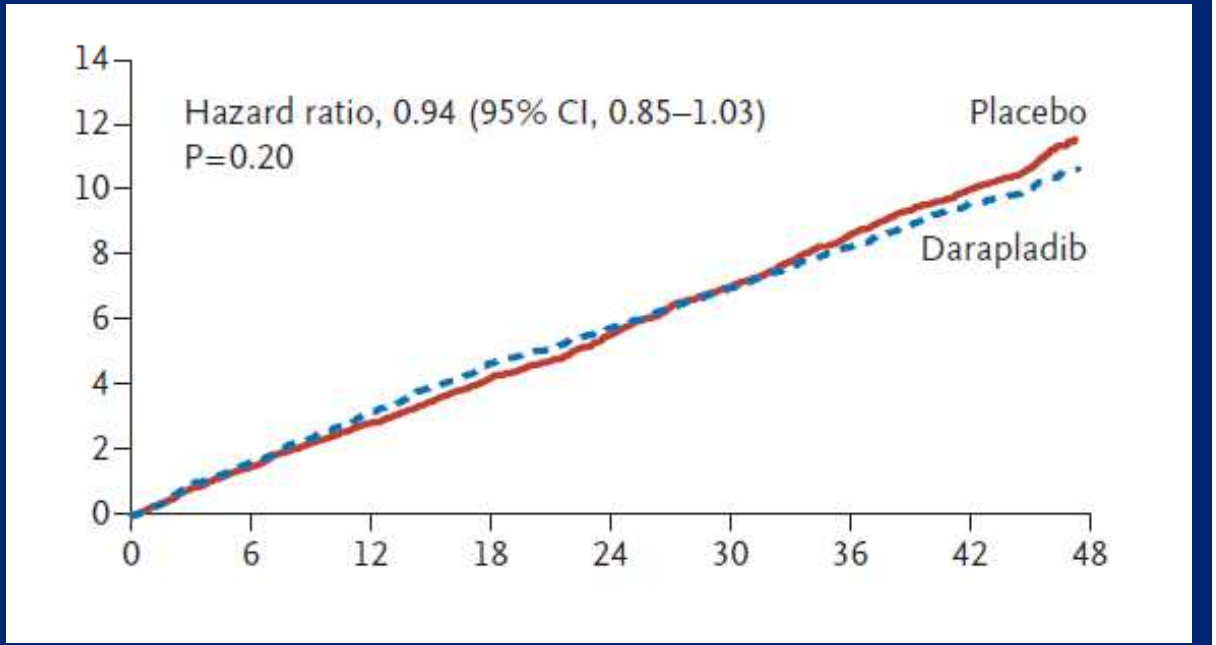
Lp-PLA_2 Studies Collaboration ,Lancet, 2010

Lp-PLA_2 - observational and trial evidence

- Increased Lp-PLA_2 activity associated with CHD.
- Large-scale randomized trials of darapladib, an Lp-PLA_2 inhibitor, showed no effect on major vascular events.



Lp-PLA_2 Studies Collaboration ,Lancet, 2010



STABILITY Investigators, NEJM, 2014

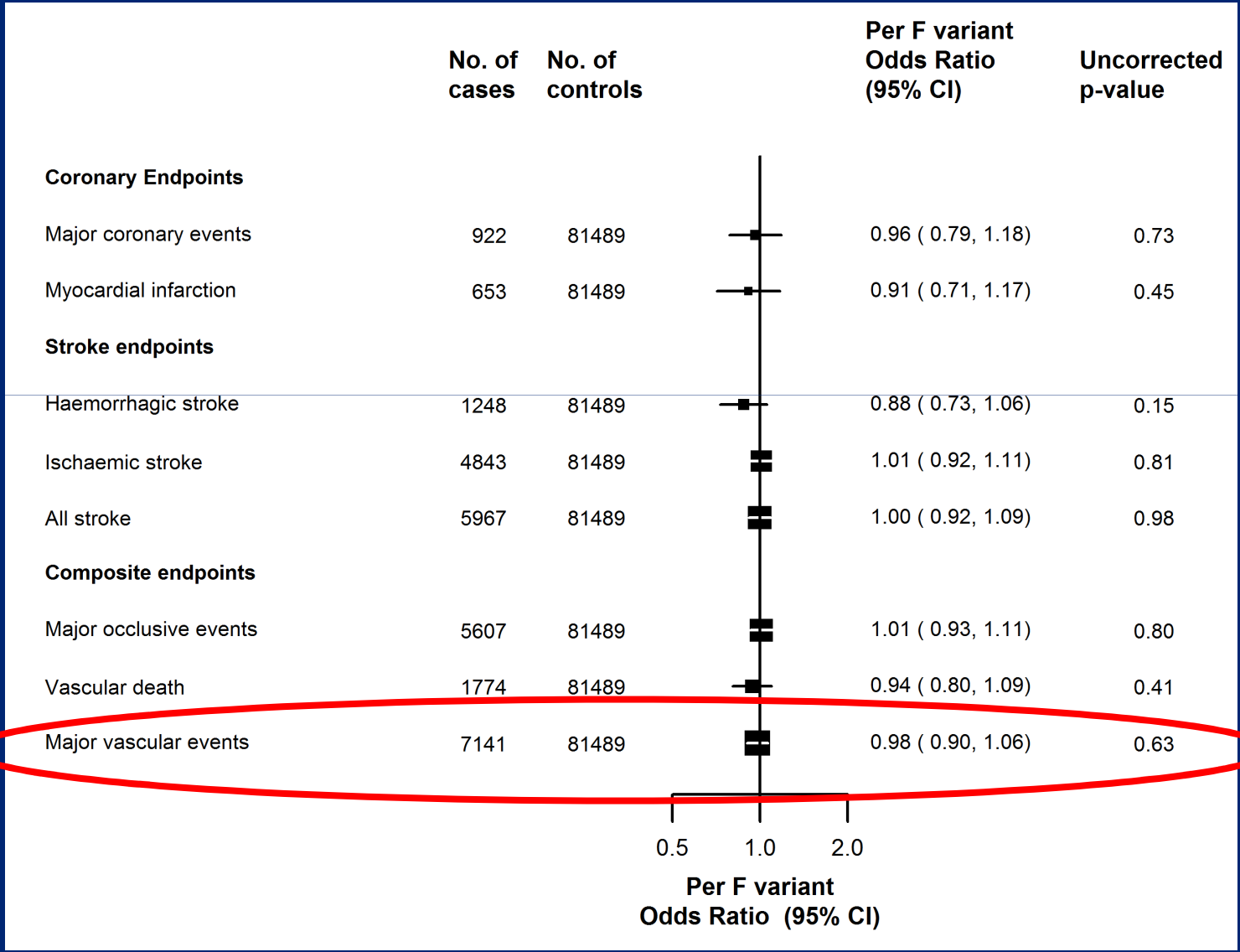
Do genetic studies agree? Lp-PLA_2 and *PLA2G7*

- A loss-of-function variant in *PLA2G7* (V279F), encoding Lp-PLA_2, results in 50% lower Lp-PLA_2 activity per copy and is common in East Asians (5%), providing a strong genetic instrument.

- V279F was genotyped in ~90,000 individuals from the China Kadoorie Biobank and tested for association with ~ 7000 major vascular events as well as a range of wider diseases (to explore other on-target effects).

Millwood et al, JACC, 2016

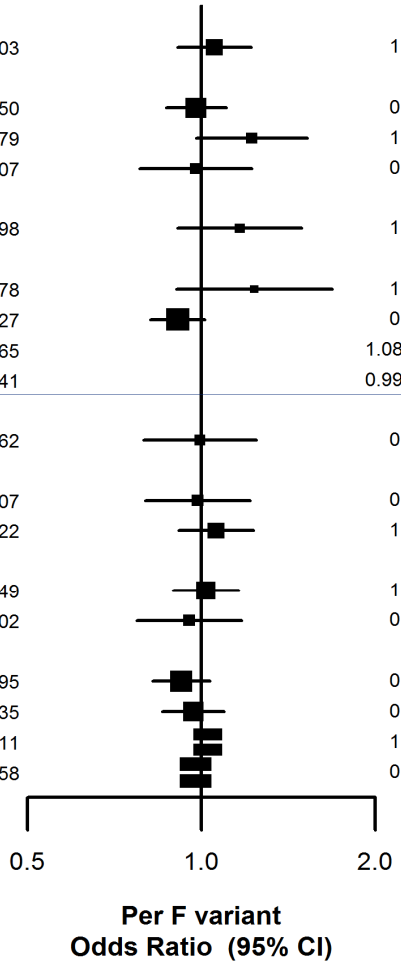
Loss-of-function mutation in the Lp-PLA$_2$ gene



Millwood et al, JACC, 2016

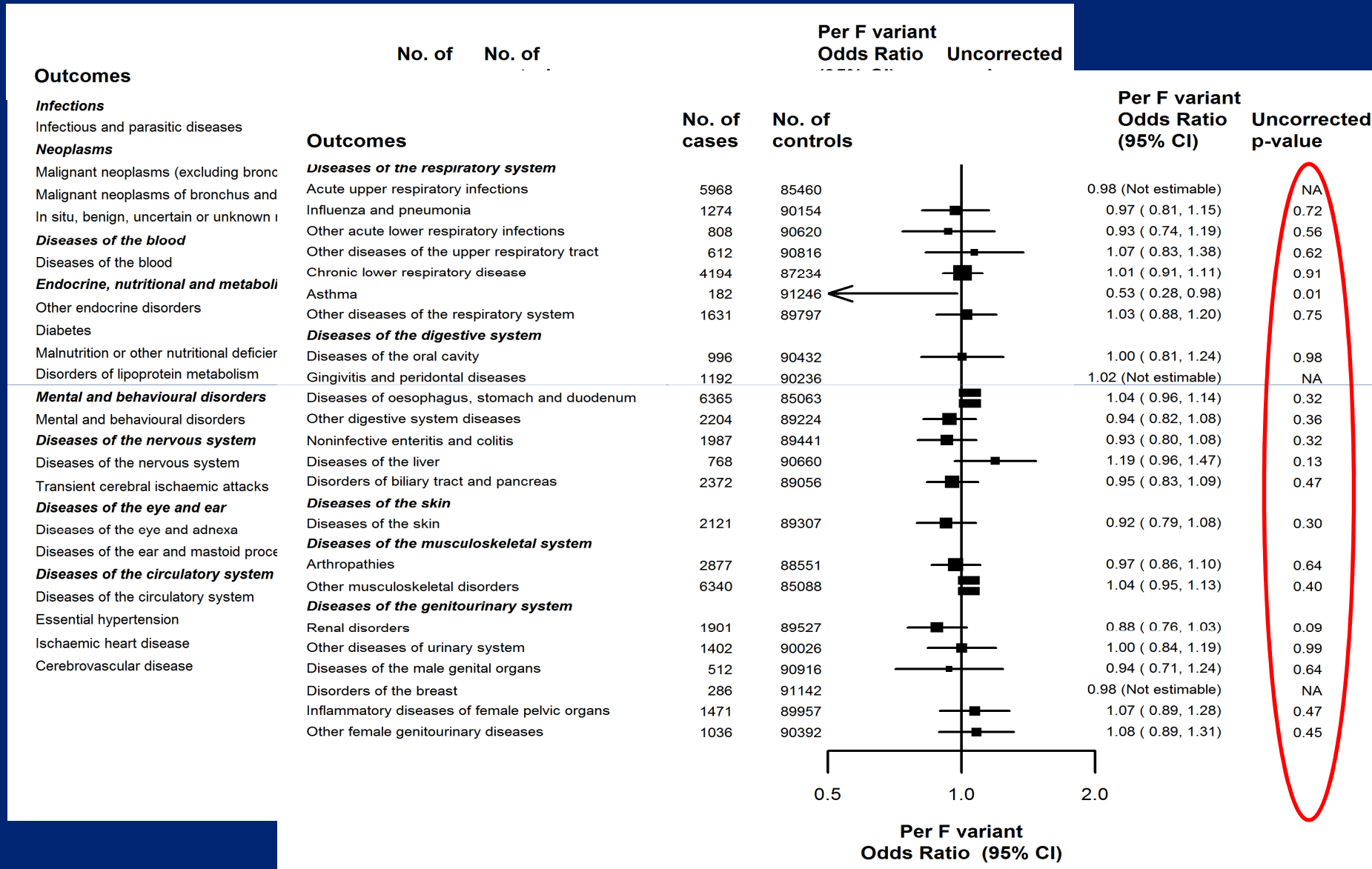
PheWAS of *PLA2G7* V279F

Outcomes	No. of cases	No. of controls	Per F variant Odds Ratio (95% CI)	Uncorrected p-value
Infections				
Infectious and parasitic diseases	2025	89403	1.05 (0.91, 1.22)	0.50
Neoplasms				
Malignant neoplasms (excluding bronchus and lung)	2778	88650	0.98 (0.87, 1.10)	0.73
Malignant neoplasms of bronchus and lung	649	90779	1.22 (0.98, 1.52)	0.10
In situ, benign, uncertain or unknown neoplasms	821	90607	0.98 (0.78, 1.22)	0.84
Diseases of the blood				
Diseases of the blood	630	90798	1.16 (0.91, 1.49)	0.26
Endocrine, nutritional and metabolic diseases				
Other endocrine disorders	350	91078	1.23 (0.90, 1.68)	0.23
Diabetes	3701	87727	0.91 (0.82, 1.01)	0.08
Malnutrition or other nutritional deficiencies	463	90965	1.08 (Not estimable)	NA
Disorders of lipoprotein metabolism	487	90941	0.99 (Not estimable)	NA
Mental and behavioural disorders				
Mental and behavioural disorders	766	90662	0.99 (0.79, 1.24)	0.96
Diseases of the nervous system				
Diseases of the nervous system	921	90507	0.98 (0.80, 1.21)	0.88
Transient cerebral ischaemic attacks	1606	89822	1.06 (0.91, 1.23)	0.45
Diseases of the eye and ear				
Diseases of the eye and adnexa	2479	88949	1.02 (0.89, 1.16)	0.78
Diseases of the ear and mastoid process	926	90502	0.95 (0.77, 1.17)	0.65
Diseases of the circulatory system				
Diseases of the circulatory system	3433	87995	0.92 (0.82, 1.03)	0.15
Essential hypertension	3393	88035	0.97 (0.86, 1.09)	0.60
Ischaemic heart disease	5517	85911	1.03 (0.94, 1.12)	0.55
Cerebrovascular disease	7570	83858	0.98 (0.90, 1.06)	0.58



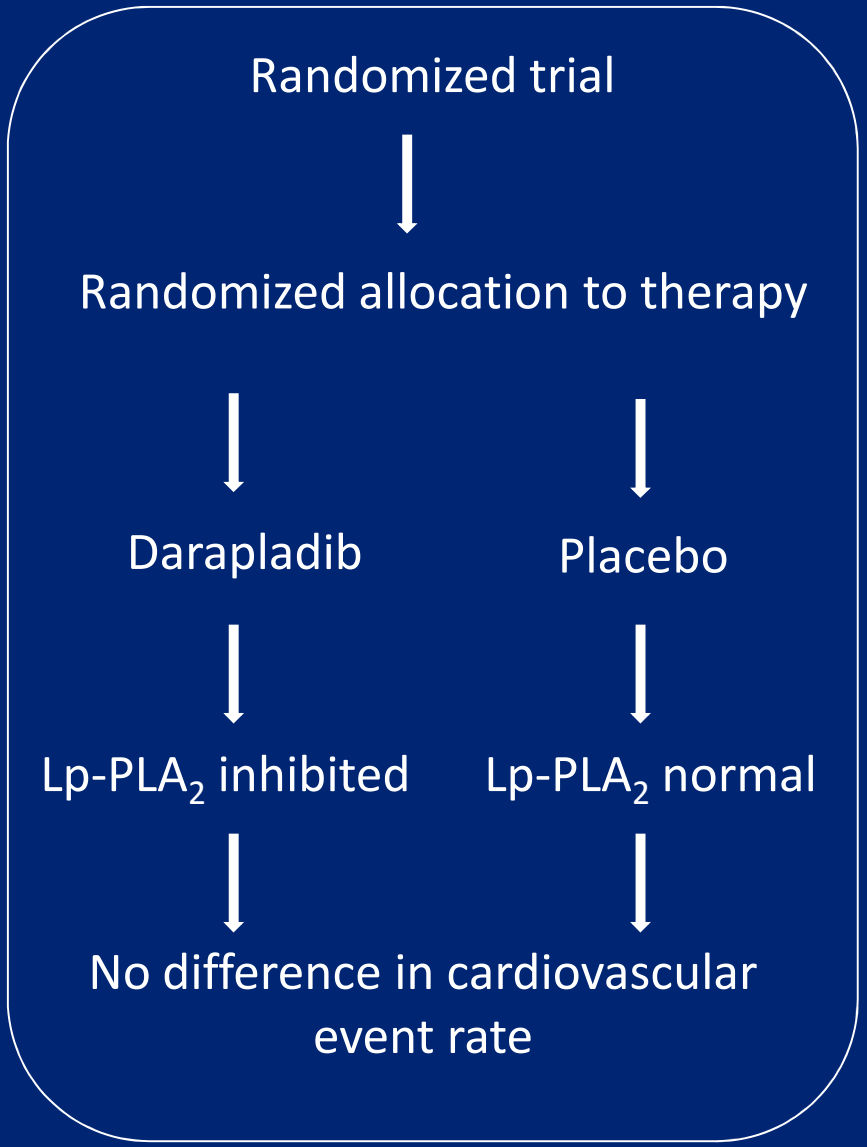
Millwood et al, IJE, 2016

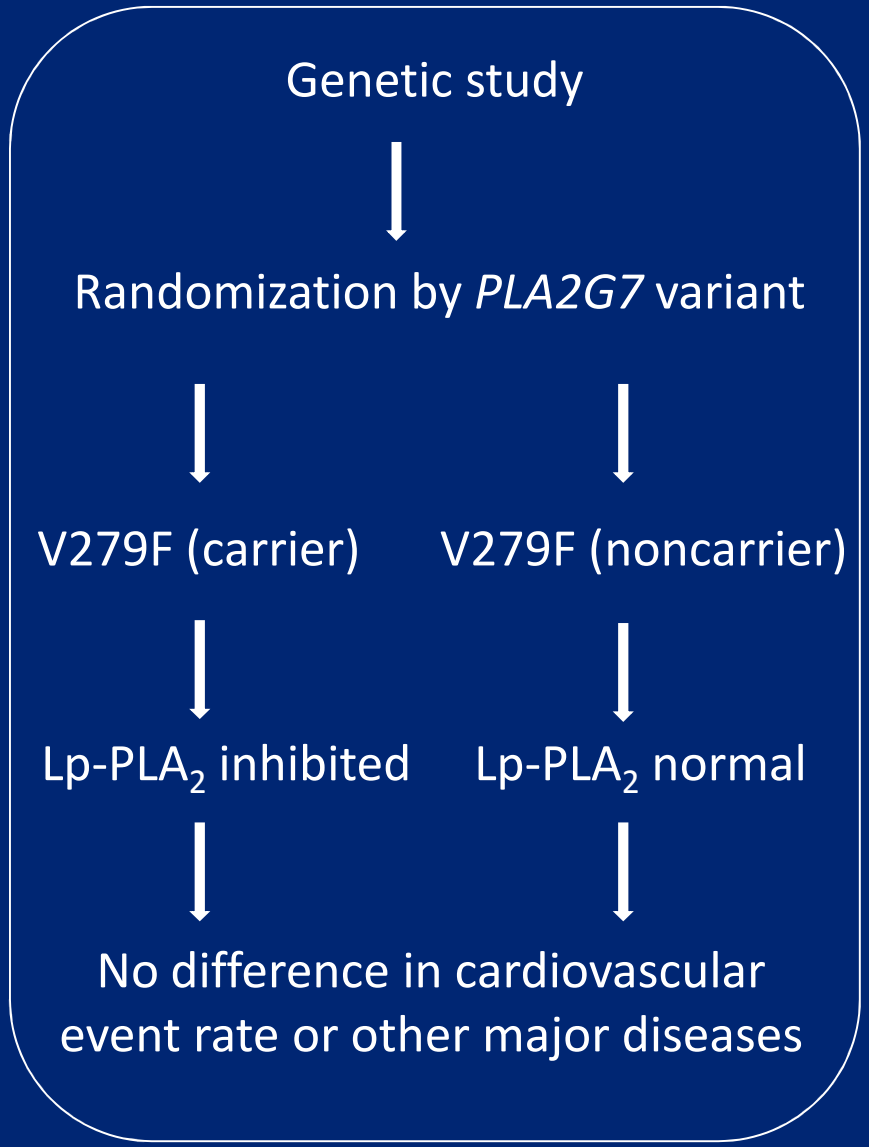
PheWAS of *PLA2G7* V279F



Millwood et al, IJE, 2016

$LpPLA_2$ – Trials vs Genetic studies





Implications

- Concordance between the effects of genetic markers and clinical trials; Lp-PLA_2 inhibition does not lower rate of MVEs. Thus, the biological mechanism isn't causal for disease.

- PheWAS indicates no large effects on other disease outcomes, suggesting no major safety concerns or other potential promising indications.

Do trials and Mendelian randomization always agree?

PCSK9 inhibitors, CHD and ischaemic stroke

PCSK9 genetic mutations to drug discovery

- ***PCSK9* gain-of-function mutations**
  - → High LDL-C
  - → Premature heart disease

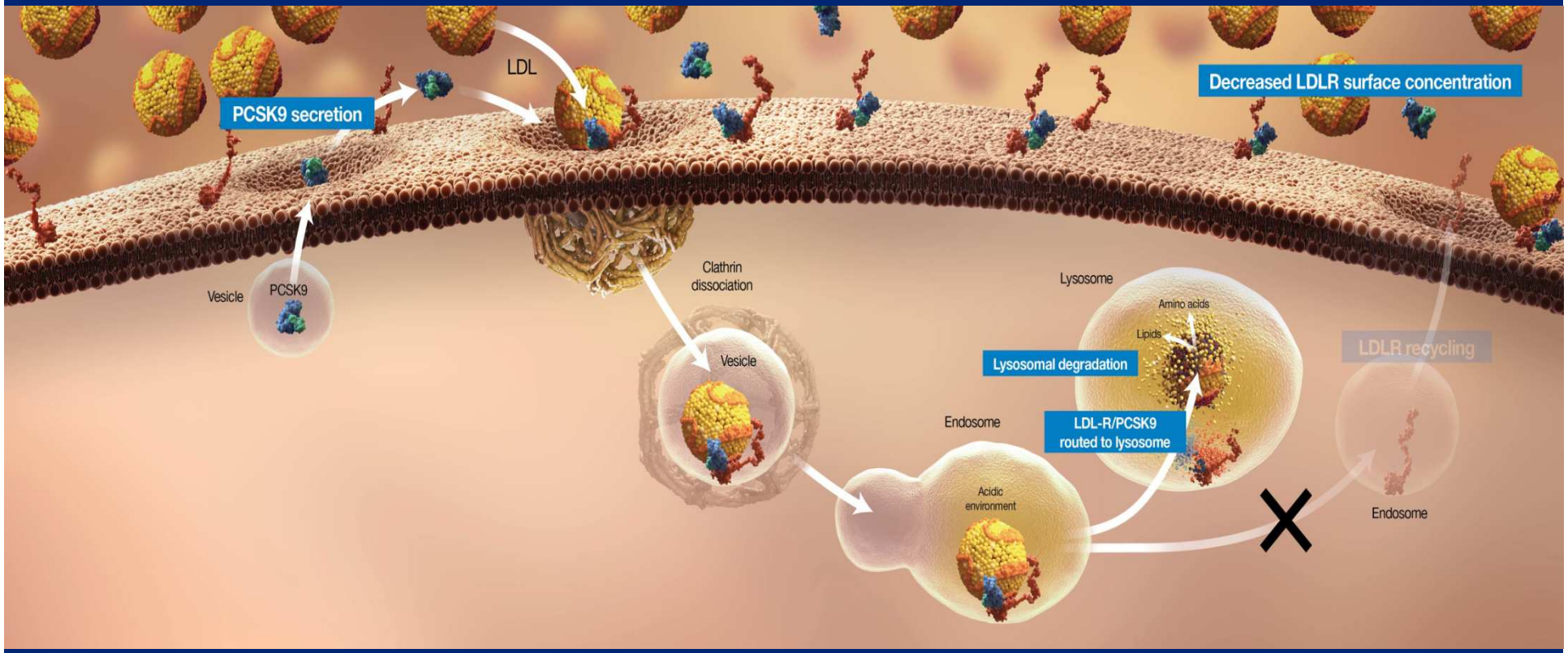
- ***PCSK9* loss-of-function mutations**
  - → Low LDL-C
  - → Reduction in CHD incidence
  - → Not associated with other detectable abnormalities

→ Development of PCSK9 inhibitors

Abifadel et al, Nat Gen, 2003
Cohen et al, Nat Gen, 2005; Cohen et al, NEJM, 2006

What does PCSK9 do?

PCSK9 regulates surface expression of LDLRs by targeting for lysosomal degradation



Qian et al, J Lipid Res, 2007
Horton et al, J Lipid Res, 2009
Zhang et al, J Biol Chem, 2007.

How might PCSK9-inhibition impact different vascular outcomes?

- Statin trials suggest that LDL-lowering lowers the risk of both CHD and of ischaemic stroke comparably.
- However, observational evidence suggests a weaker effect of LDL-C on ischaemic stroke than on CHD.
- Prior to very recent Phase 3 studies of PCSK9 inhibition, there was no reliable direct evidence for the impact of PCSK9 on vascular outcomes.

Impact of PCSK9 inhibition on vascular outcomes FOURIER – a Phase 3 clinical trial

Outcome	Evolocumab (N=13,784)	Placebo (N=13,780)	Hazard Ratio (95% CI)	P Value*
	no. of patients (%)			
Primary end point: cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization	1344 (9.8)	1563 (11.3)	0.85 (0.79–0.92)	<0.001
Key secondary end point: cardiovascular death, myocardial infarction, or stroke	816 (5.9)	1013 (7.4)	0.80 (0.73–0.88)	<0.001
Myocardial infarction	468 (3.4)	639 (4.6)	0.73 (0.65–0.82)	<0.001
Hospitalization for unstable angina	236 (1.7)	239 (1.7)	0.99 (0.82–1.18)	0.89
Stroke	207 (1.5)	262 (1.9)	0.79 (0.66–0.95)	0.01
Ischemic	171 (1.2)	226 (1.6)	0.75 (0.62–0.92)	
Hemorrhagic	29 (0.21)	25 (0.18)	1.16 (0.68–1.98)	
Unknown	13 (0.09)	14 (0.10)	0.93 (0.44–1.97)	

Evolocumab resulted in a 27% risk reduction in myocardial infarction and a 25% risk reduction in ischaemic stroke.

Sabatine et al, NEJM 2017

What do the genetics suggest?

- International collaboration of studies examining the genetics of ischaemic stroke (IS), involving ~10,000 IS cases from 12 studies (about 50% with TOAST subtyping).
- Effects of *PCSK9* variants on IS and IS subtypes in the METASTROKE genome-wide meta-analysis were estimated.
- Estimates of the effects of *PCSK9* variants on LDL-C and CHD risk were obtained from the Global Lipids Consortium and CARDIoGRAMPlusC4D Consortium respectively.

Hopewell et al, EHJ, In Press

PCSK9 R46L and risk of vascular disease

The R46L loss-of-function variant (~1.5% frequency in Europeans), is associated with lower PCSK9 levels, and ~0.5 mmol/L lower LDL-C levels (as well as small effects on other biomarkers).

Hopewell et al, EHJ, In Press

PCSK9 R46L and risk of vascular disease

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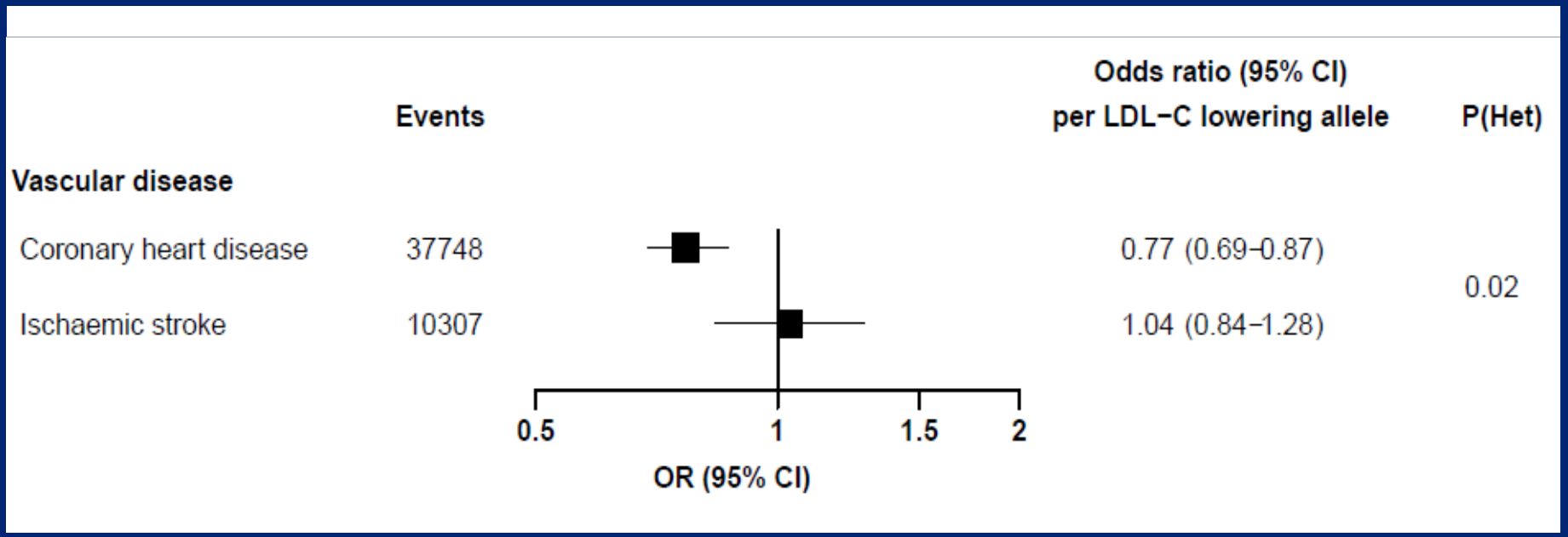
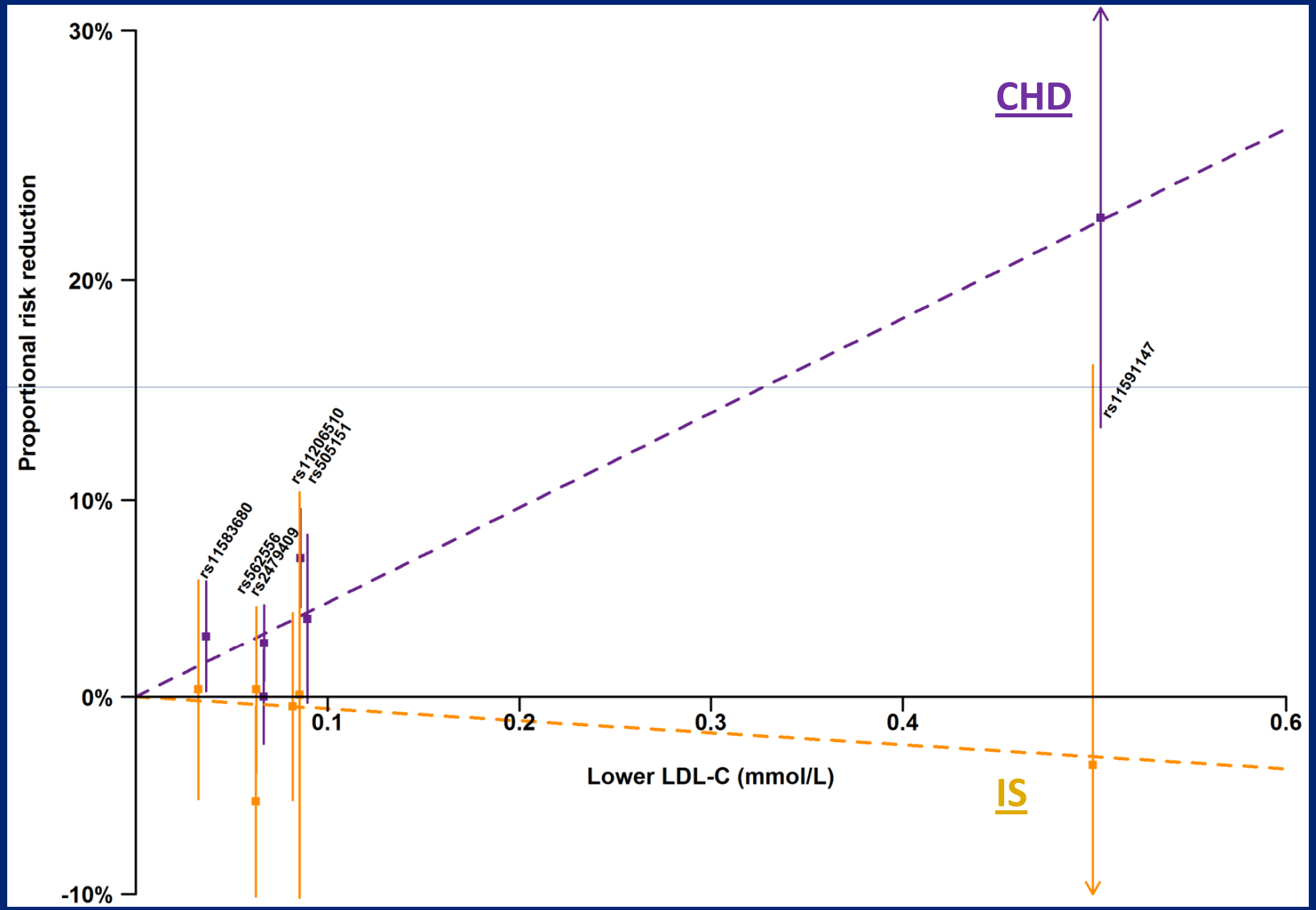


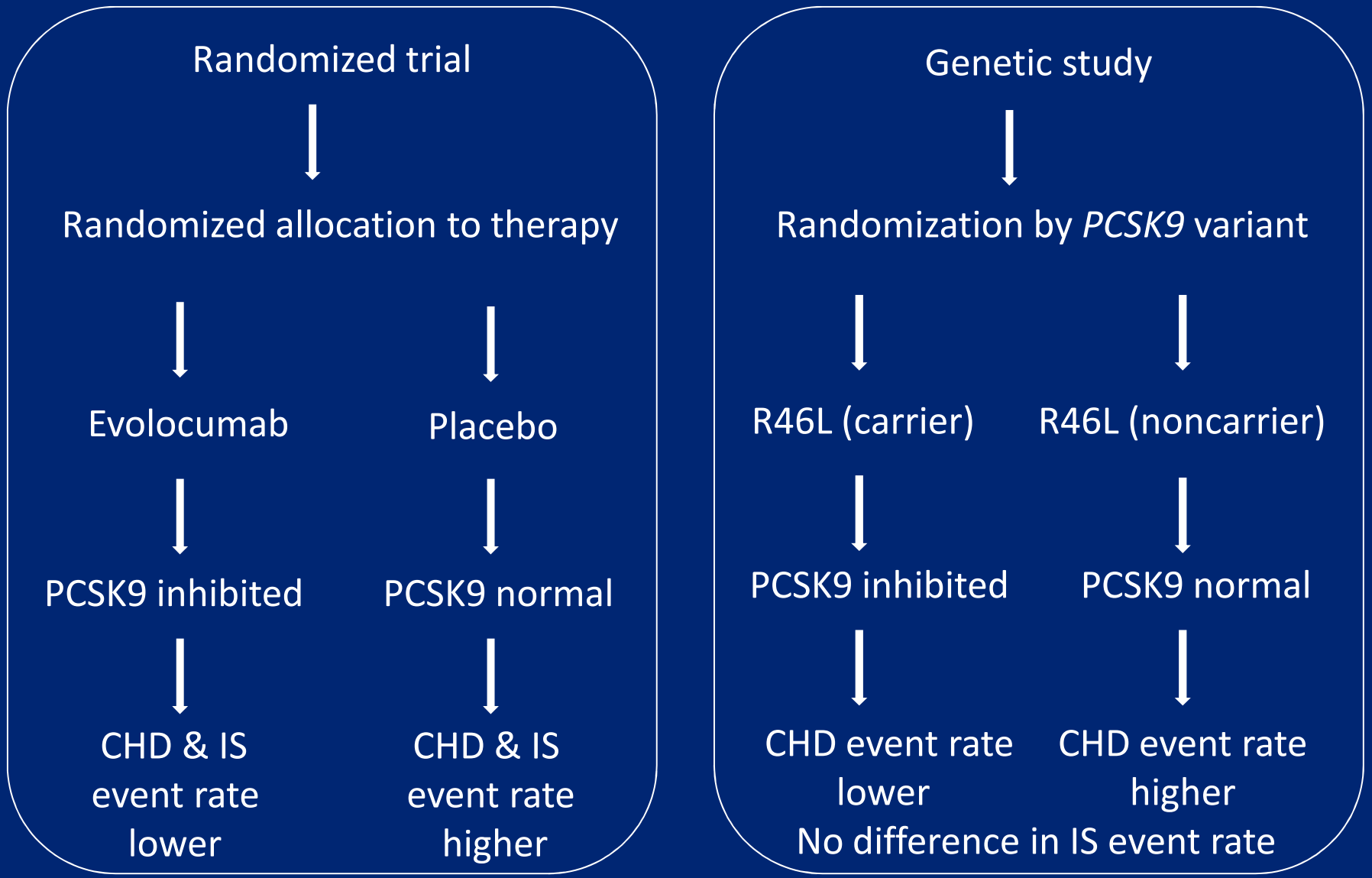
Hopewell et al, EHJ, In Press

Impact of *PCSK9* variants on LDL-C lowering and vascular risk



Hopewell et al, EHJ, in press

PCSK9 – Trials vs Genetic studies



Implications

- *PCSK9* variants that produce life-long lower LDL-C levels, and that are associated with lower risk of CHD, appear to have a significantly weaker (if any) effect on the risk of IS.

- By comparison, PCSK9-inhibitor therapy (similarly to statin and ezetimibe) reduces the risk of CHD and IS comparably.

- Findings illustrate potential limitations with the use of Mendelian randomization to predict the effects of novel therapeutic interventions on different health outcomes.

Mendelian randomization
Take home messages

- Mendelian randomization is a powerful tool that can help to elucidate the causal relevance of therapeutic mechanisms and biomarkers.

- Mendelian randomization studies can be complex and interpretation challenging, thus findings should be considered alongside other sources of evidence.

- Statistical approaches are available that may facilitate better understanding of Mendelian randomization studies and the potential impact of pleiotropy.

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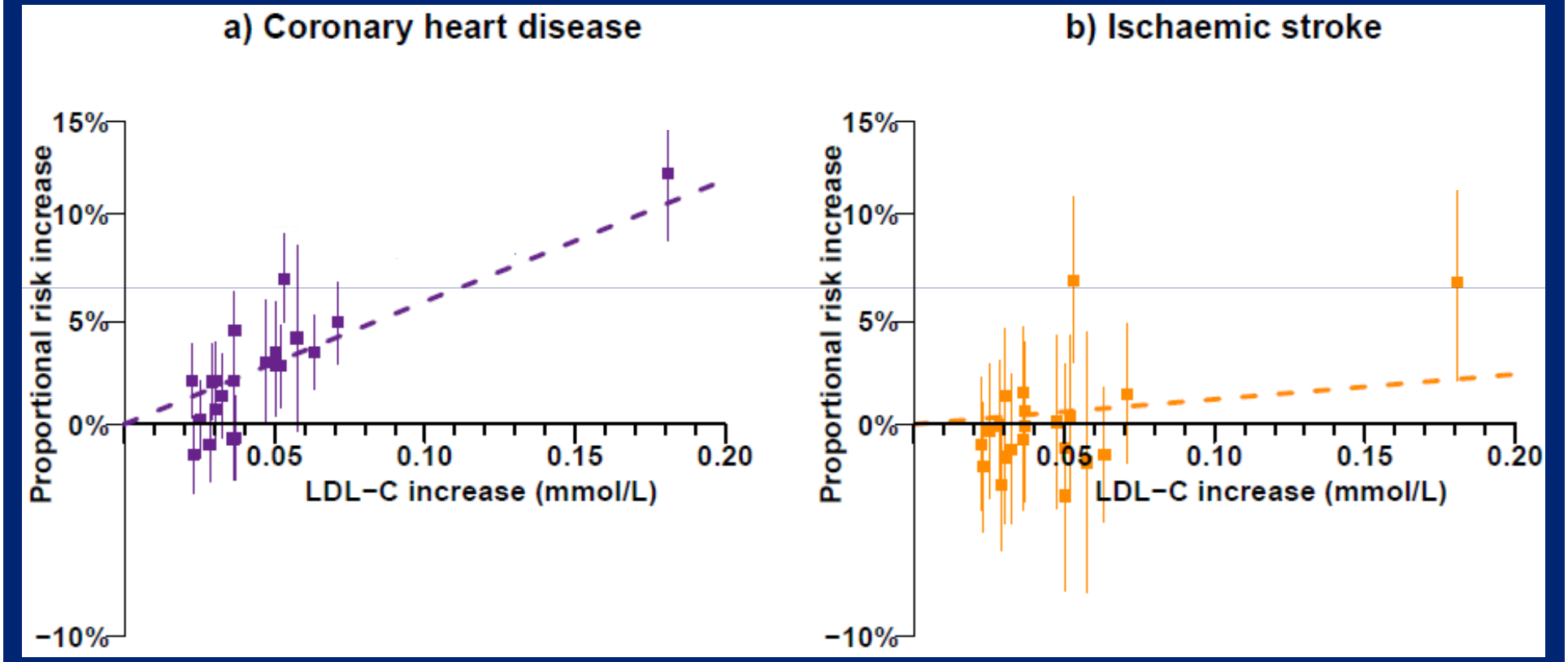
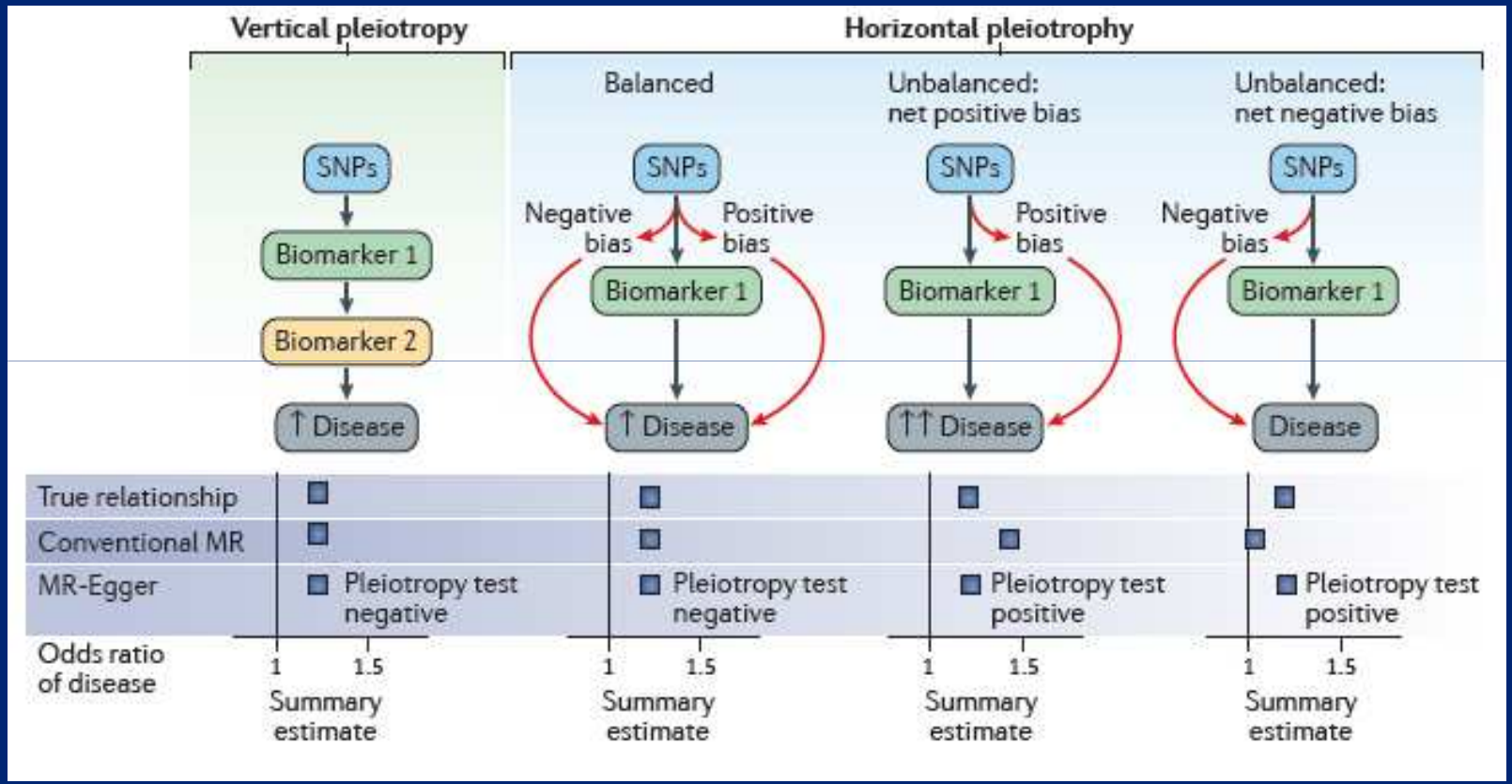
Back-up slides

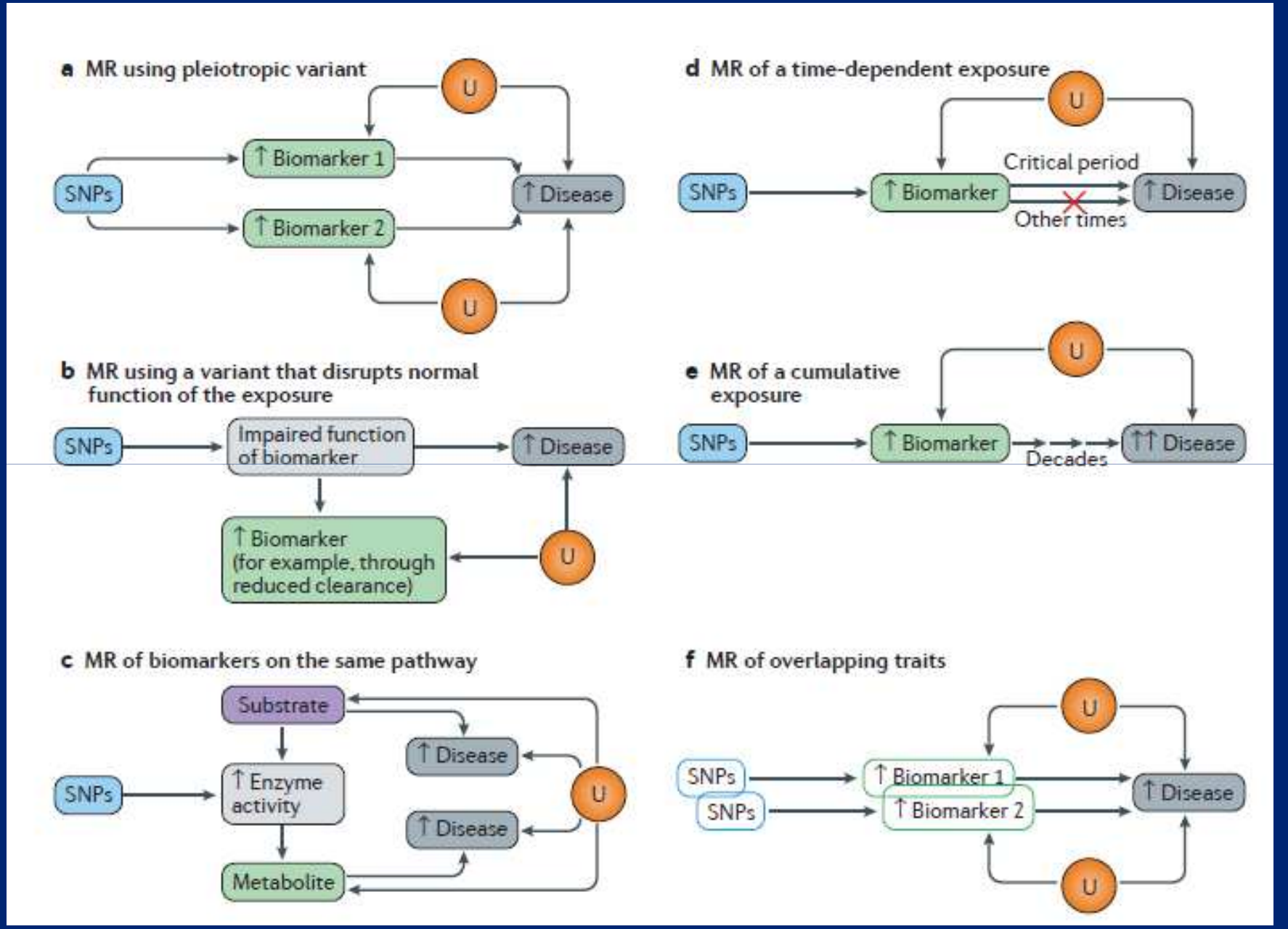
MR of specific LDL-C lowering variants also suggests weaker effect on IS than on CHD risk



Observe heterogeneity between effects on CHD and on IS



Holmes et al, Nat Rev Cardio, 2017



Holmes et al, Nat Rev Cardio, 2017

Statins & *PCSK9*

Statins increase PCSK9 (limiting their impact)

↓

PCSK9 inhibitors decrease PCSK9 expression

↓

Statin in the presence of PCSK9 inhibition should be more effective!

Statins and fibrates induce increased PCSK9 expression, thus PCSK9 inhibition could induce robust LDL reduction as add-on therapy.