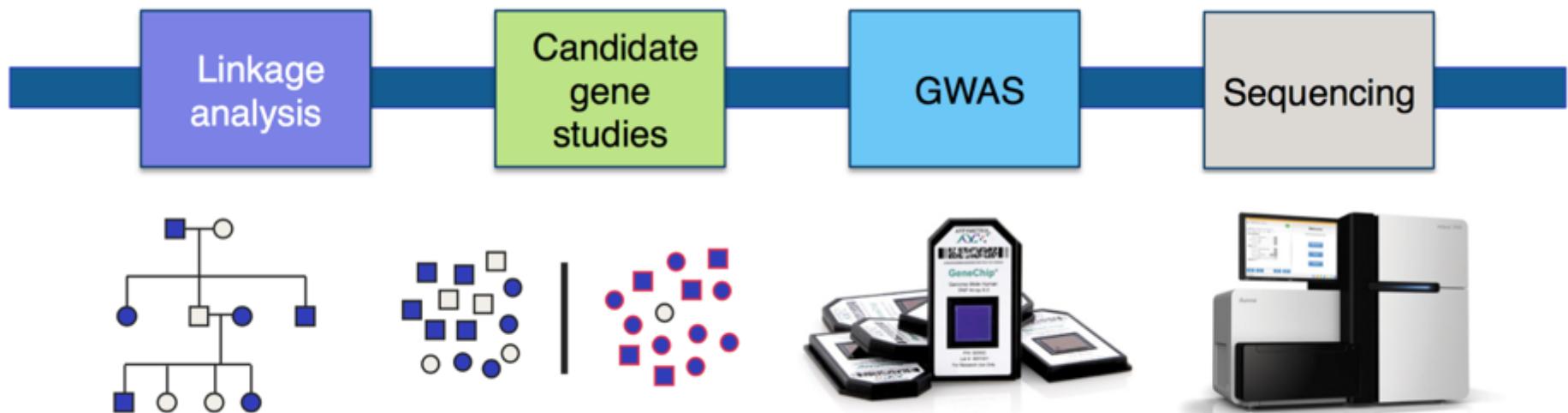
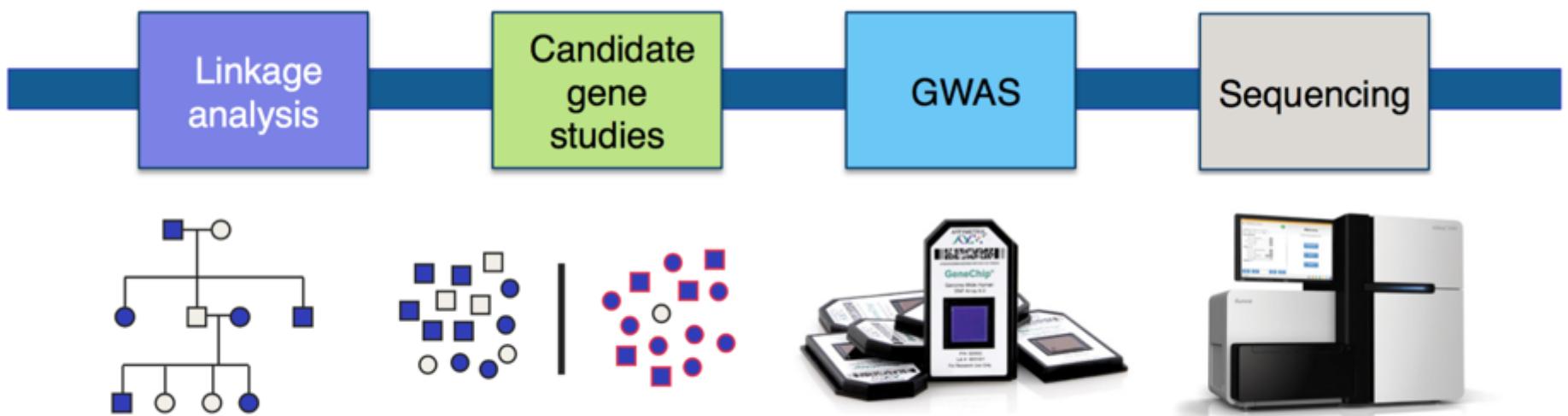


# What have we learned in the field of cardiovascular genetics?



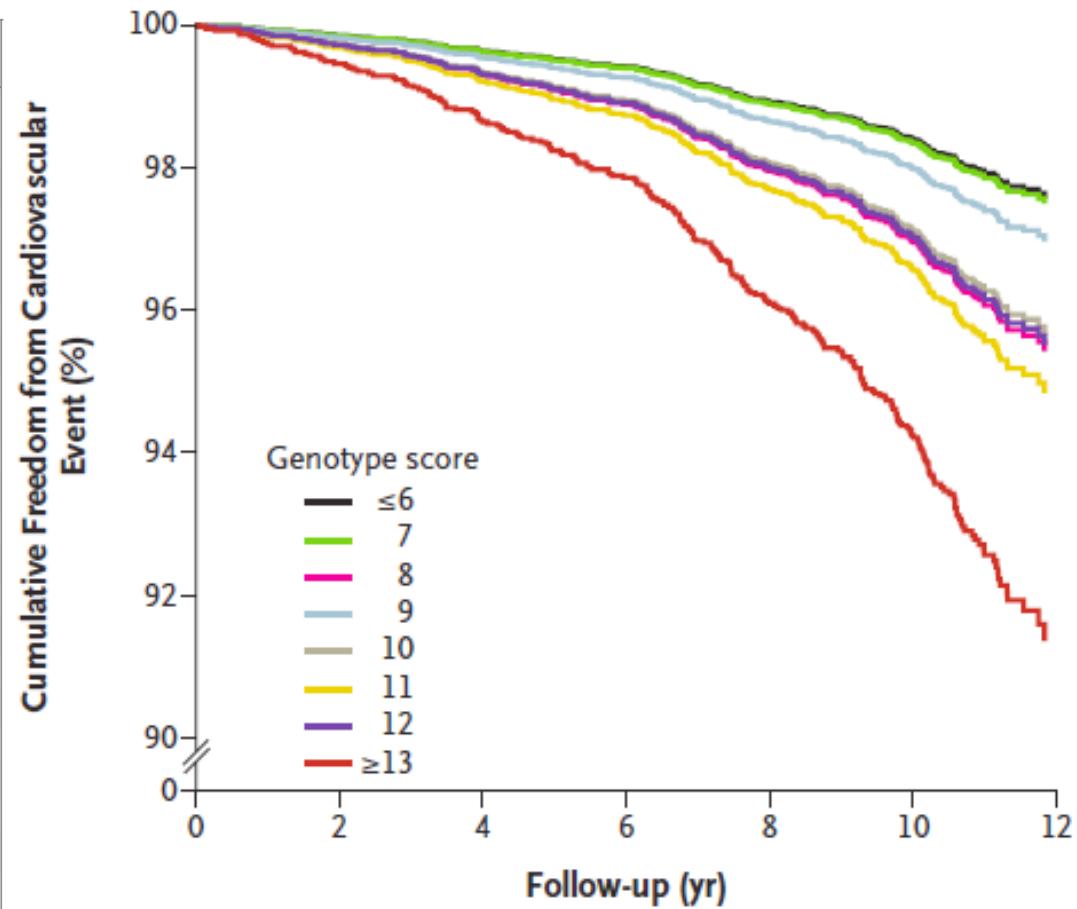
# Prediction?



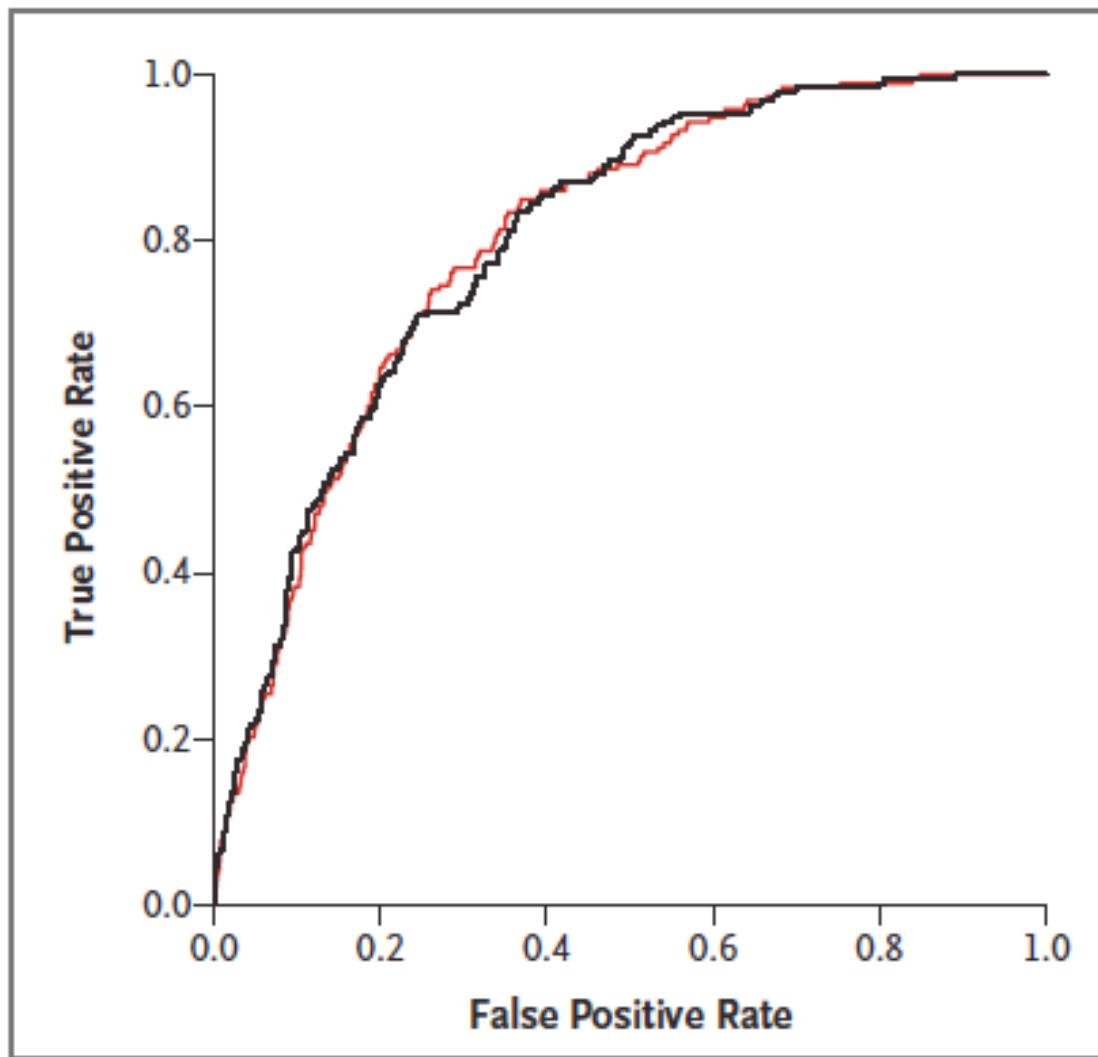
# 9 SNPs associations with CVD

**Table 4.** Multivariable Analysis of the Association between Genotype Score and the Time to First Cardiovascular Event.\*

	Multivariable-Adjusted Hazard Ratio (95% CI)	P Value
Age, per SD	1.77 (1.52–2.07)	<0.001
Male sex	1.61 (1.20–2.17)	0.002
Parent or sibling with history of myocardial infarction	1.52 (1.17–1.97)	0.002
Cholesterol, per SD		
LDL	1.13 (0.99–1.29)	0.08
HDL	0.75 (0.61–0.91)	0.003
Log triglycerides, per SD	0.87 (0.73–1.04)	0.12
Blood pressure, per SD		
Systolic	1.29 (1.08–1.54)	0.005
Diastolic	1.16 (0.97–1.38)	0.11
Body-mass index, per SD	1.09 (0.94–1.25)	0.26
Diabetes mellitus	1.47 (1.02–2.13)	0.04
Status of cigarette smoking		<0.001†
Former versus never	1.17 (0.85–1.59)	
Current versus never	2.00 (1.41–2.83)	
Log C-reactive protein, per SD	1.14 (0.99–1.30)	0.06
Drug therapy		
Lipid-lowering	1.29 (0.63–2.64)	0.48
Antihypertensive	1.46 (1.08–1.97)	0.01
Genotype score, per single unfavorable allele	1.15 (1.07–1.24)	<0.001



# But does not aid risk prediction



**Figure 2.** Receiver-Operating-Characteristic (ROC) Curves for Incident Myocardial Infarction, Ischemic Stroke, or Death from Coronary Heart Disease during 10-Year Follow-up.

The curves are based on risk-prediction models incorporating 14 clinical covariates that either included the genotype score (black line) or did not include the genotype score (red line). The C statistic (area under the ROC curve) for total cardiovascular events was the same (0.80) for both risk models.

MENU ▾

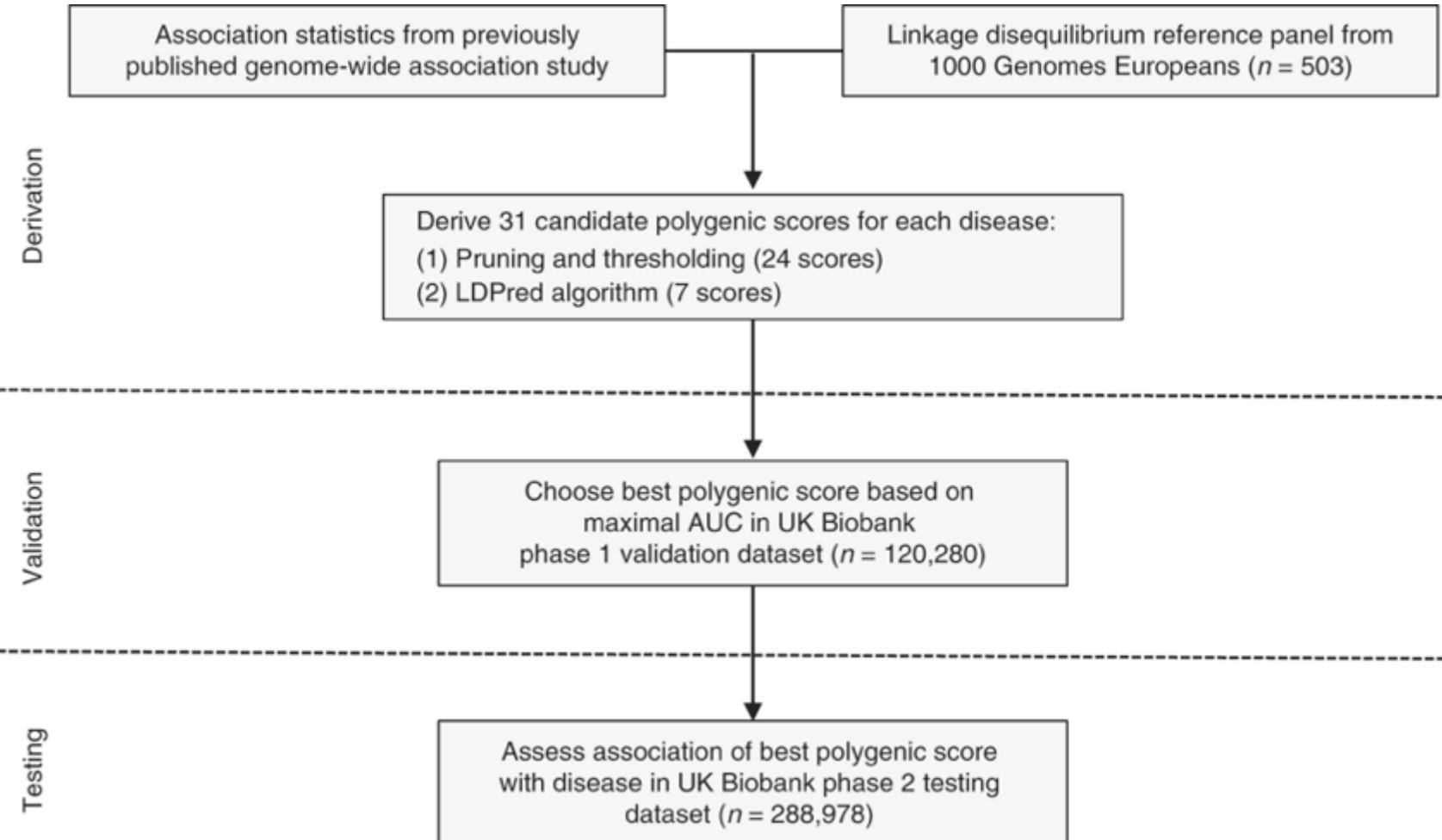
Letter | Published: 13 August 2018

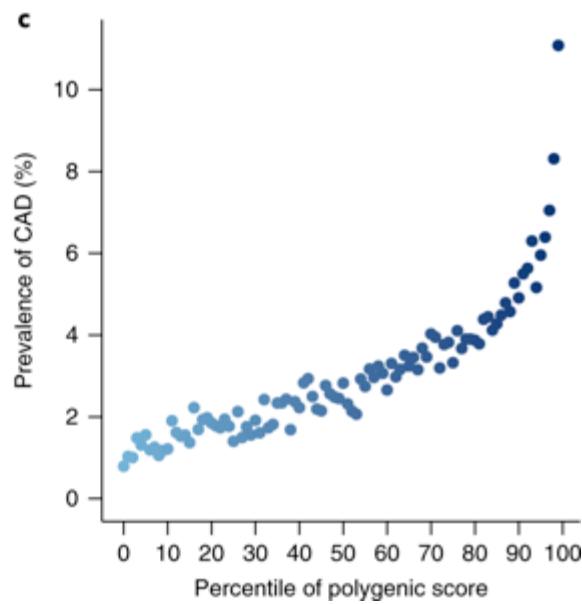
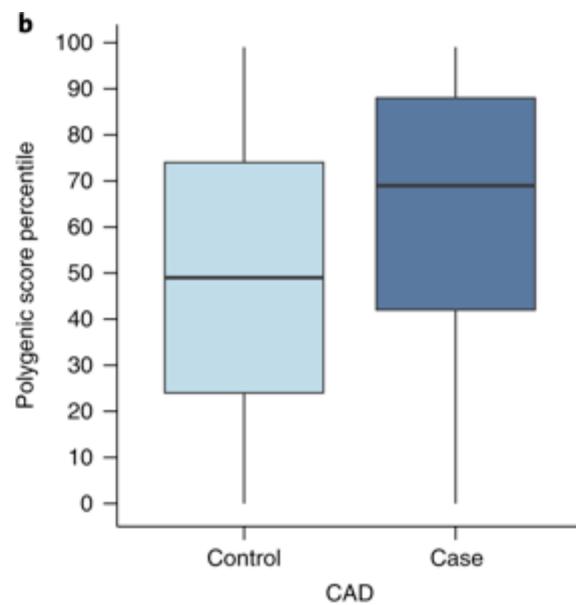
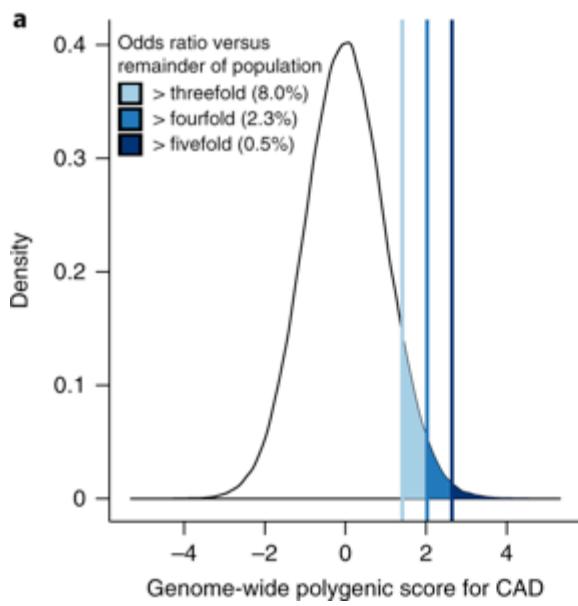
# Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations

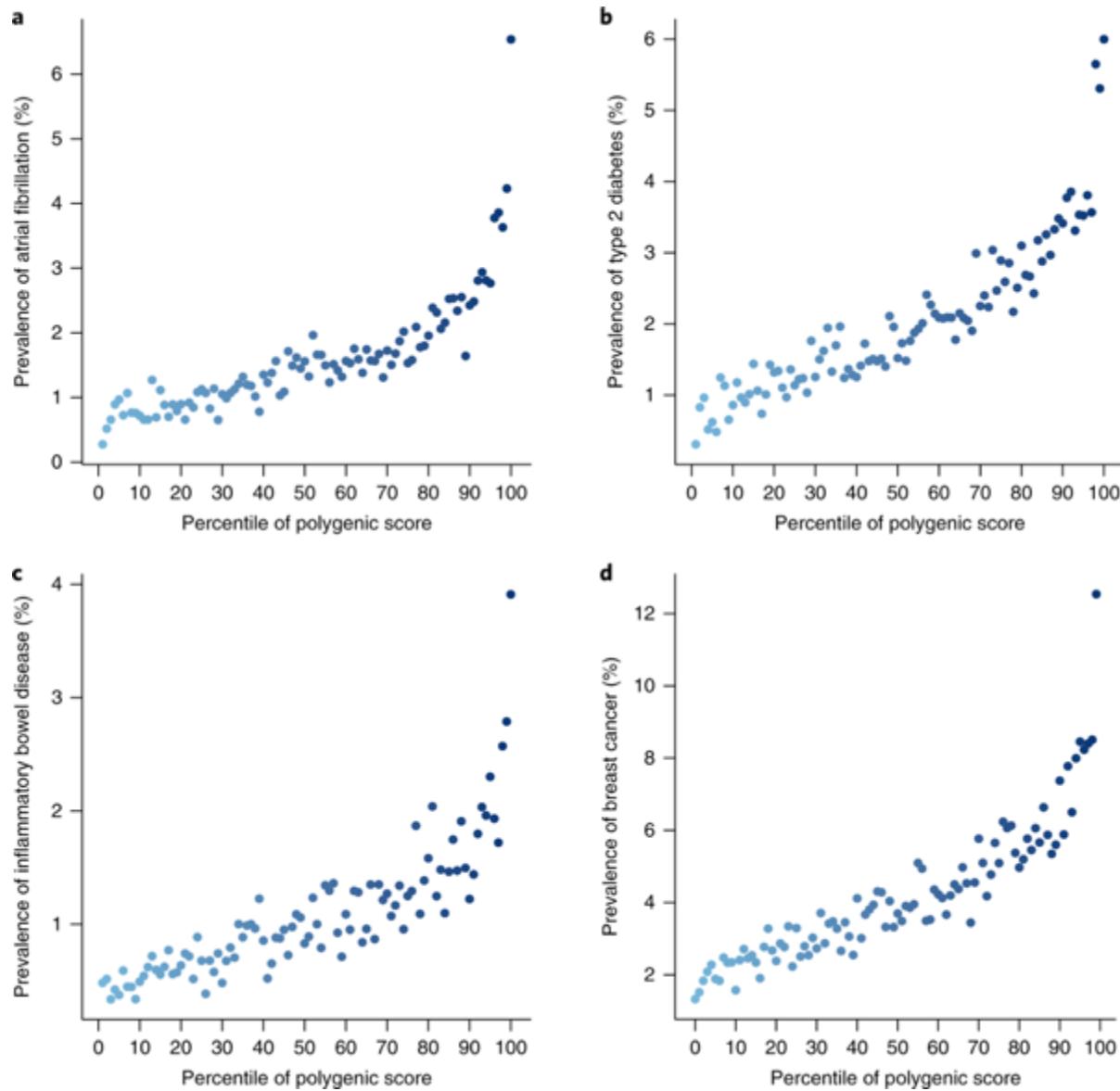
Amit V. Khera, Mark Chaffin, Krishna G. Aragam, Mary E. Haas, Carolina Roselli, Seung Hoan Choi, Pradeep Natarajan, Eric S. Lander, Steven A. Lubitz, Patrick T. Ellinor & Sekar Kathiresan✉

*Nature Genetics* **50**, 1219–1224 (2018) | Download Citation ↴

# Include millions of variants of small effects







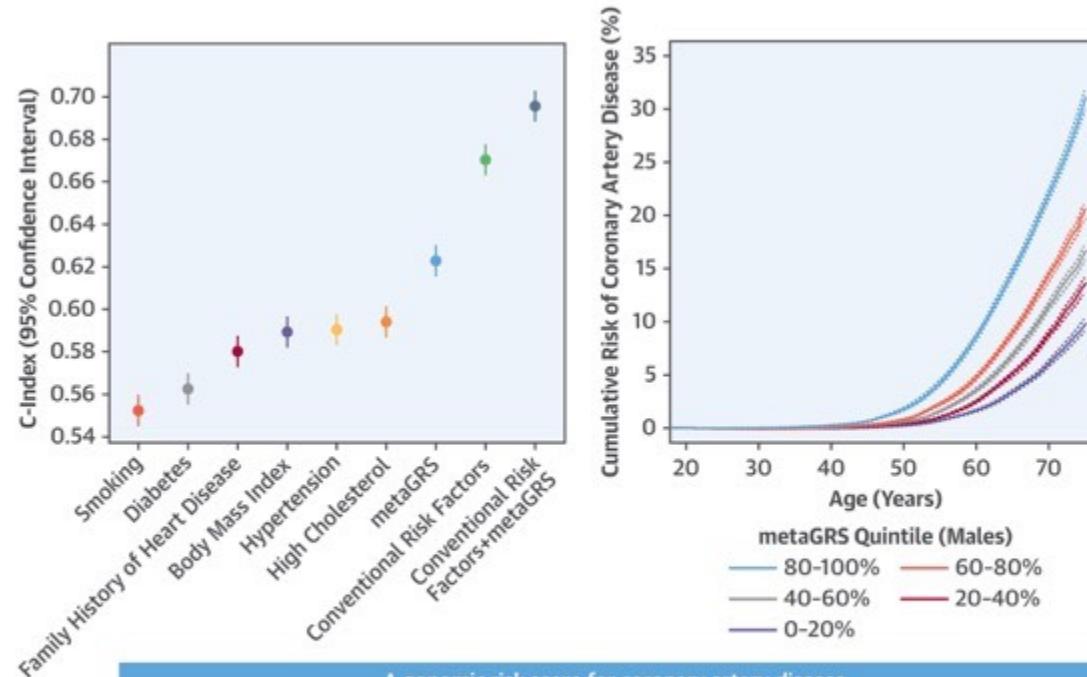
High GPS definition	Individuals in testing dataset ( n )	% of individuals
<b>Odds ratio ≥3.0</b>		
CAD	23,119/288,978	8.0
Atrial fibrillation	17,627/288,978	6.1
Type 2 diabetes	10,099 288,978	3.5
Inflammatory bowel disease	9,209 288,978	3.2
Breast cancer	2,369/157,895	1.5
Any of the five diseases	57,115/288,978	19.8
<b>Odds ratio ≥4.0</b>		
CAD	6,631/288,978	2.3
Atrial fibrillation	4,335/288,978	1.5
Type 2 diabetes	578/288,978	0.2
Inflammatory bowel disease	2,297/288,978	0.8
Breast cancer	474/157,895	0.3
Any of the five diseases	14,029/288,978	4.9
<b>Odds ratio ≥5.0</b>		
CAD	1,443/288,978	0.5
Atrial fibrillation	2,020 288,978	0.7
Type 2 diabetes	144/288,978	0.05
Inflammatory bowel disease	571/288,978	0.2
Breast cancer	158/157,895	0.1
Any of the five diseases	4,305/288,978	1.5

## Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults

## Implications for Primary Prevention

Michael Inouye, Gad Abraham, Christopher P. Nelson, Angela M. Wood, Michael J. Sweeting, Frank Dudbridge, Florence Y. Lai, Stephen Kaptoge, Marta Brozynska, Tingting Wang, Shu Ye, Thomas R. Webb, Martin K. Rutter, Ioanna Tzoulaki, Riyaz S. Patel, Ruth J.F. Loos, Bernard Keavney, Harry Hemingway, John Thompson, Hugh Watkins, Panos Deloukas, Emanuele Di Angelantonio, Adam S. Butterworth, John Danesh, Nilesh J. Samani and for the UK Biobank CardioMetabolic Consortium CHD Working Group

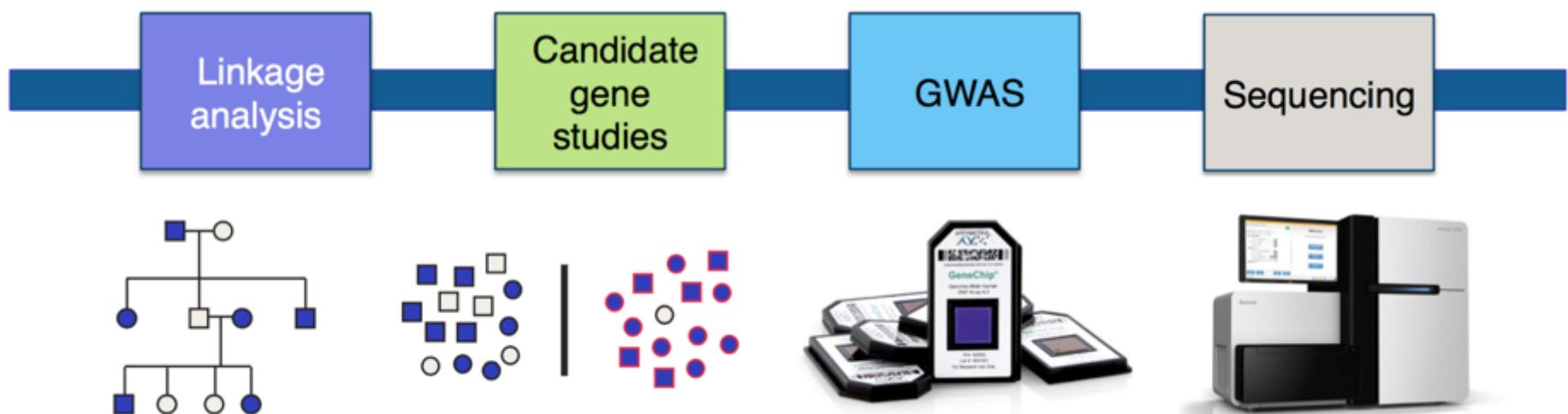
## CENTRAL ILLUSTRATION: Genomic Risk Score for Coronary Artery Disease



A genomic risk score for coronary artery disease  
Greater association with future coronary artery disease than any single conventional risk factor  
Independent of yet complements conventional risk factors  
Provides meaningful lifetime risk estimates of coronary artery disease  
Quantifiable at or before birth and shows potential for risk screening in early life

Inouye, M. et al. J Am Coll Cardiol. 2018;72(16):1883-93.

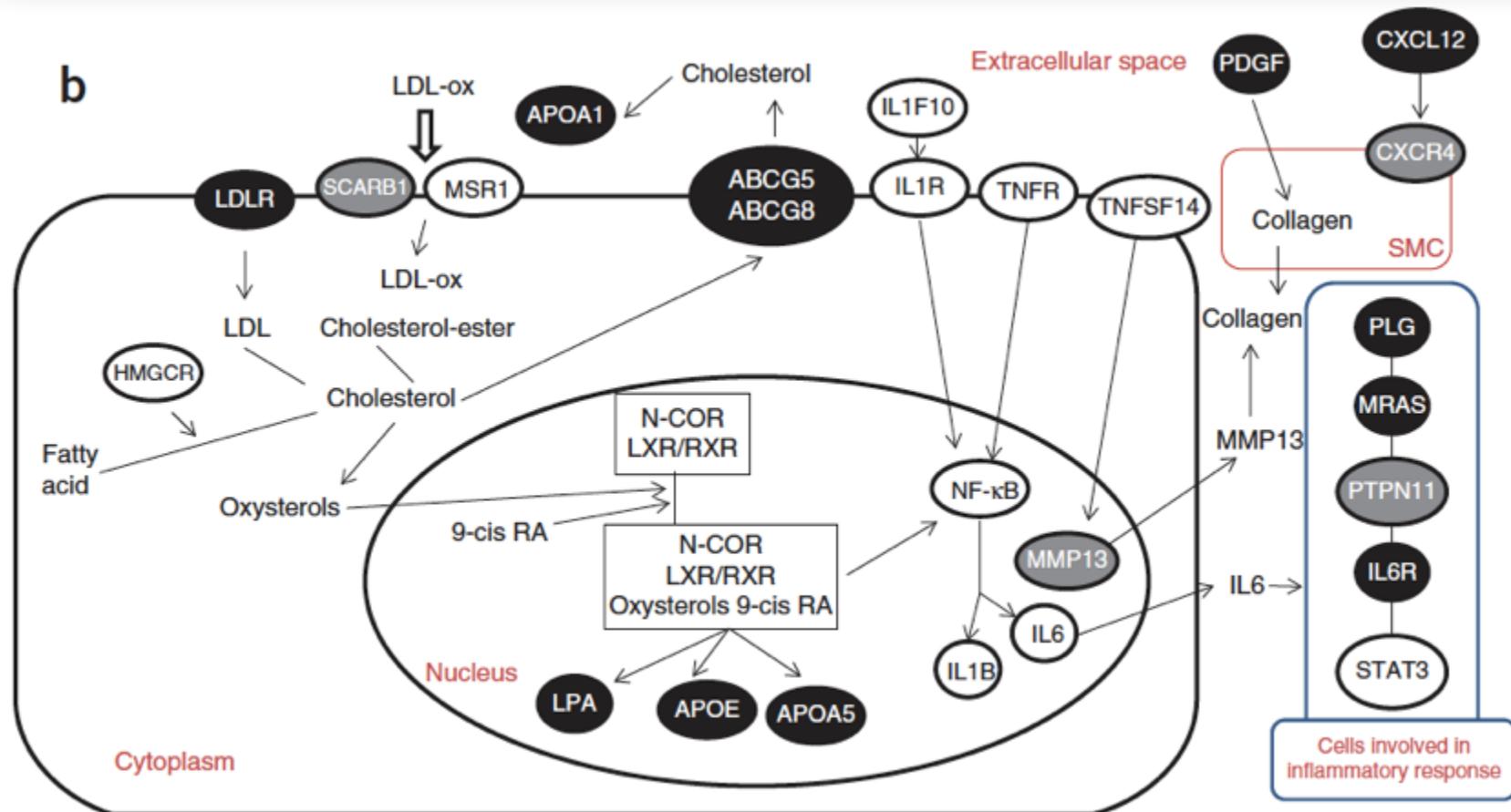
# Biological mechanisms?



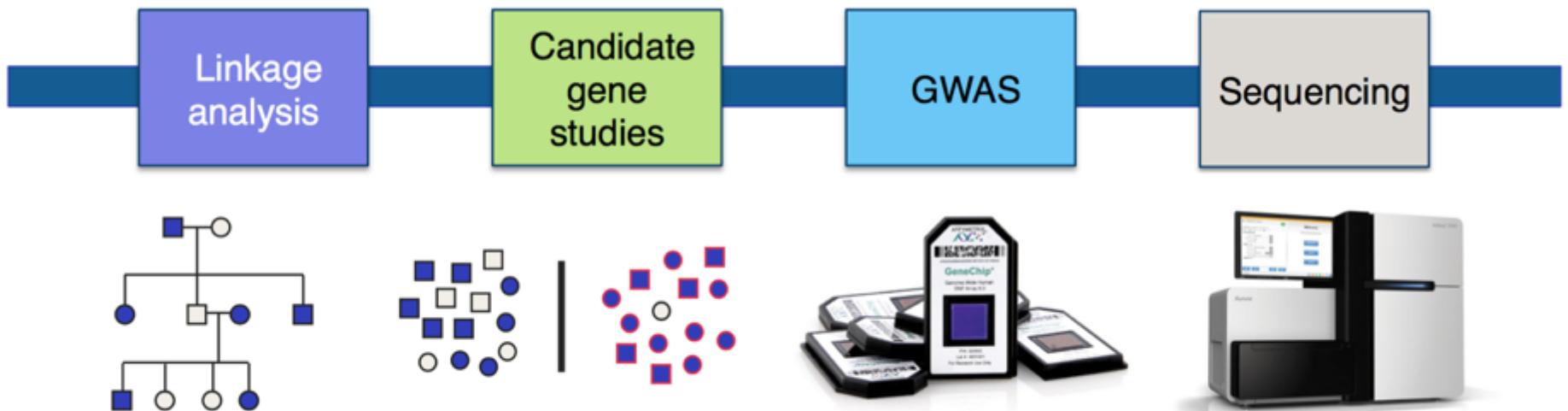
# Large-scale association analysis identifies new risk loci for coronary artery disease

63,746 cases and 130,681 controls: 46 CAD loci

The CARDIoGRAMplusC4D Consortium<sup>1</sup>



# Causality: Mendelian Randomization?



## APOLIPOPROTEIN E ISOFORMS, SERUM CHOLESTEROL, AND CANCER

SIR.—It is unclear whether the relation between low serum cholesterol levels and cancer<sup>1</sup> is causal. In many studies occult tumour may have depressed cholesterol levels though in others the relation was found when serum cholesterol had been measured many years before the cancer was diagnosed. The relation is probably not explained by diet, because in the Seven Countries Study cohorts with widely different diets and corresponding differences in mean cholesterol levels experienced similar mean cancer rates.<sup>2,3</sup> On the other hand, within each region cancer incidence was higher in men with a serum cholesterol in the lowest part of the cholesterol distribution for that country.<sup>3</sup> Thus, naturally low cholesterol levels are sometimes associated with increased cancer risk.<sup>1,3</sup>

Differences in the aminoacid sequence of apolipoprotein E (apo E) are major determinants of differences in plasma cholesterol levels within a population. Apo E has a key role in the clearance of cholesterol from plasma.<sup>4</sup> The synthesis of apo E is under the control of three independent alleles, located at a single gene locus, coding for the major isoforms E-2, E-3, and E-4 with respective population frequencies of about 8, 77, and 15%.<sup>5</sup> The homozygous E-3/E-3 is the most common phenotype encountered and E-2/E-2 is the least common. From apo E-2 to apo E-3, one cysteine residue is replaced by arginine, and from apo E-3 to apo E-4 another cysteine residue is replaced. As a result the avidity of apo E containing lipoproteins for lipoprotein receptors increases from apo E-2 to apo E-3 to apo E-4. In several populations,<sup>6-8</sup> including the Finns and the Japanese (Dr G. Utermann, personal communication), the gradient in serum cholesterol levels in the population is associated with a gradient in apo E phenotype, E-2 being associated with lower serum low-density lipoprotein and total cholesterol levels than E-3 and E-4. Thus, if a naturally low cholesterol favours tumour

growth, then subjects with the E-2/E-2 or E-2/E-3 phenotype should have an increased risk of cancer.

Unlike most other indices of lipid metabolism, apolipoprotein aminoacid sequences are not disturbed by disease, and the apo E phenotype found in a patient will have been present since birth. A comparison of apo E phenotypes in cancer patients with those in matched controls might thus shed light on the relation between low cholesterol and cancer. If it is causal then the E-2 allele should be more common among patients and E-3 and E-4 more common among controls. On the other hand, equal distribution of apo E phenotypes among cases and controls would suggest that the association between low cholesterol and cancer is spurious. Measurement of apo E phenotype by isoelectric focusing of plasma is a routine determination in lipid laboratories; epidemiologists interested in cholesterol and cancer should include it in their studies.

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Agricultural University,  
6703 BC Wageningen, Netherlands

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1. McMichael AJ, Jensen OM, Parkin DM, Zaridze DG. Dietary and endogenous cholesterol and human cancer. *Epidemiol Rev* 1984; **6**: 192-216.
2. Keys A, Aravanis C, Blackburn H, et al. Serum cholesterol and cancer mortality in the seven countries study. *Am J Epidemiol* 1985; **121**: 870-83.
3. Katan MB. Effects of cholesterol-lowering diets on the risk for cancer and other non-cardiovascular diseases. In: Nestel PJ, et al, eds. Atherosclerosis VII: Proceedings of the Seventh International Atherosclerosis Symposium. Amsterdam: Elsevier, 1986.
4. Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. *Science* 1981; **212**: 628-35.
5. Utermann G, Steinmetz A, Weber W. Genetic control of human apolipoprotein E polymorphism: comparison of one- and two-dimensional techniques of isoprotein analysis. *Hum Genet* 1982; **60**: 344-51.
6. Utermann G, Kindermann I, Kaffarnik H, Steinmetz A. Apolipoprotein E phenotypes and hyperlipidemia. *Hum Genet* 1984; **65**: 232-36.
7. Robertson FW, Cumming AM. Effects of Apoprotein E Polymorphism on Serum Lipoprotein Concentration. *Arteriosclerosis* 1985; **5**: 283-92.
8. Utermann G. Genetic polymorphism of apolipoprotein E: impact on plasma lipoprotein metabolism. In: Crepaldi G, et al, eds. Diabetes, obesity and hyperlipidemias III. Amsterdam: Elsevier, 1985: 1-28.

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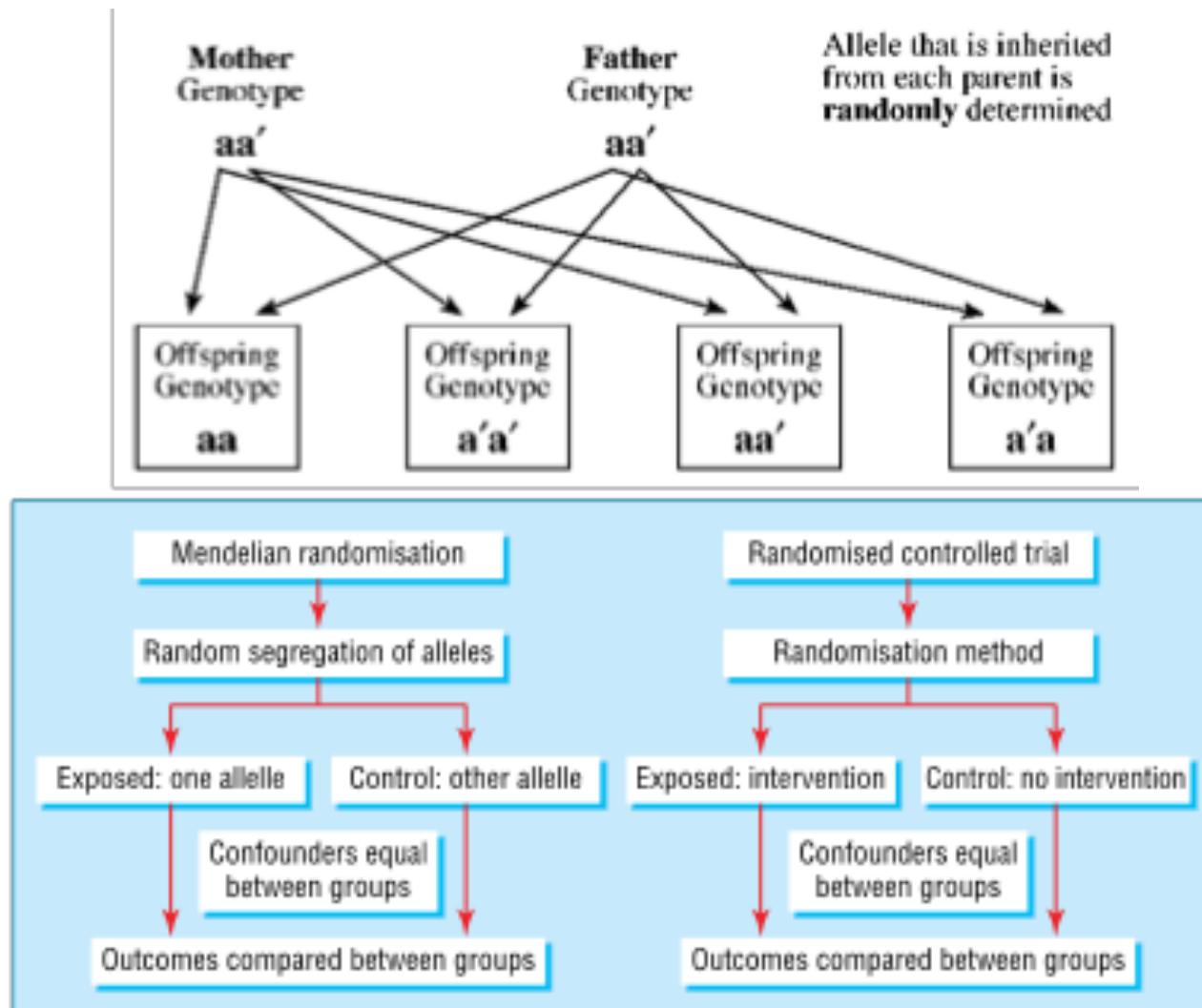
cardiovascular diseases. In: Nestel PJ, et al, eds. *Atherosclerosis VII: Proceedings of the Seventh International Atherosclerosis Symposium*. Amsterdam: Elsevier, 1986.

4. Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. *Science* 1981; **212**: 628-35.
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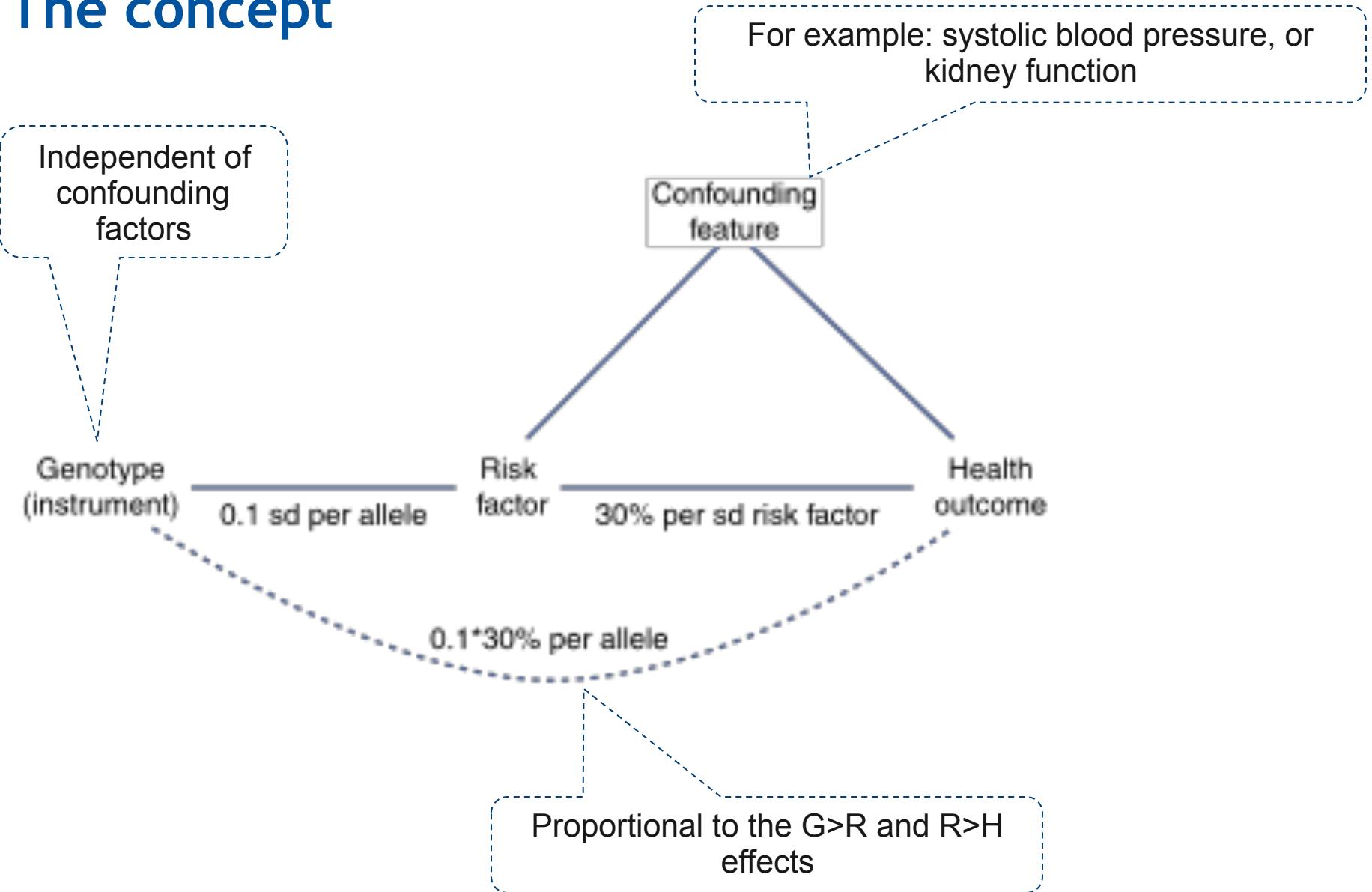


Gregor Johann Mendel - 20 July 1822 – 6 January 1884

# Alleles are randomly assigned: a “natural” treatment in a “natural” randomized trial



# The concept



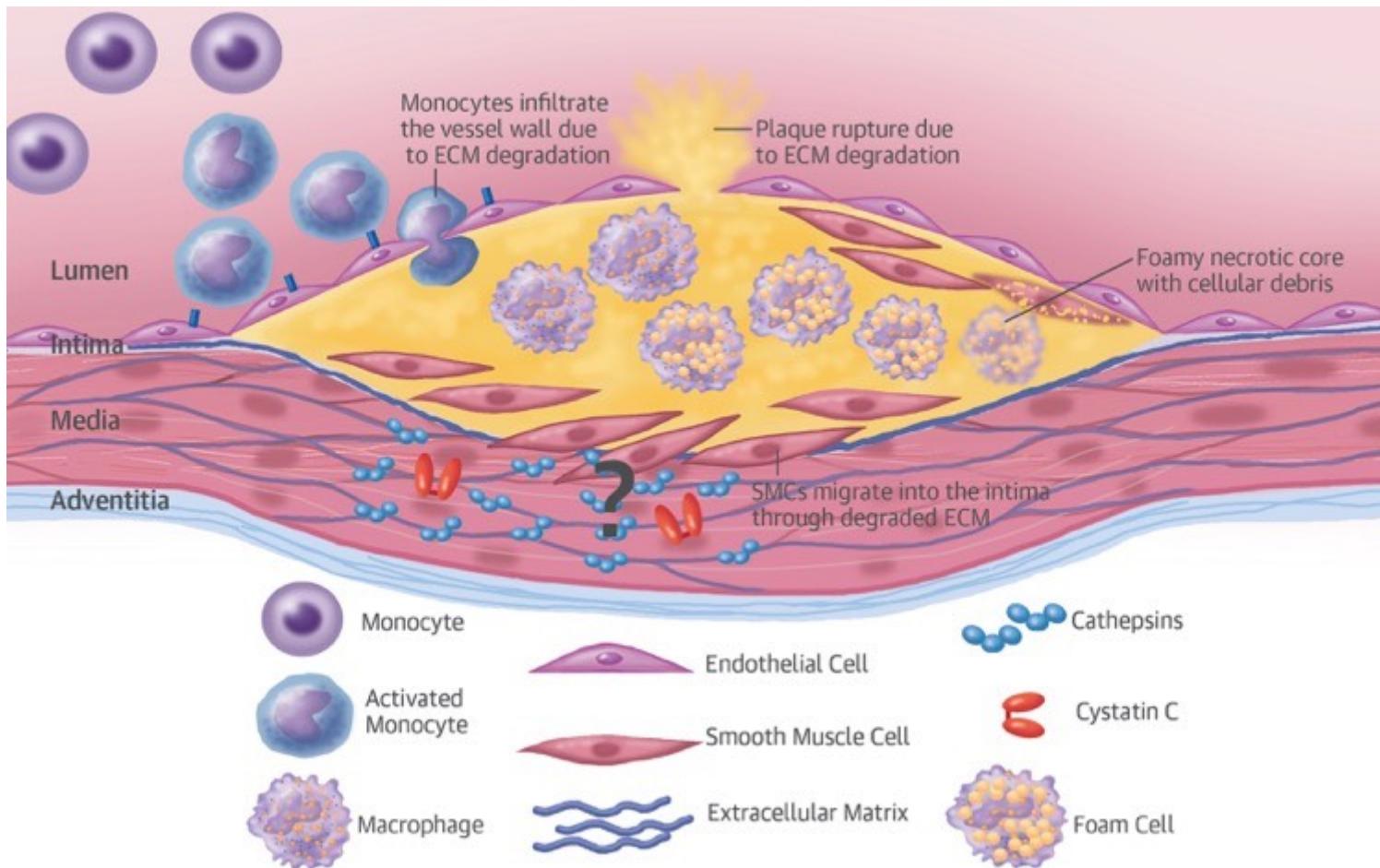
## Cystatin C and Cardiovascular Disease: A Mendelian Randomization Study

Sander W. van der Laan MSc <sup>a</sup>, <sup>b</sup>, Tove Fall PhD <sup>b</sup>, <sup>c</sup>, Aicha Soumaré PhD <sup>c</sup>, Alexander Teumer PhD <sup>c</sup>, Sanaz Sedaghat MSc <sup>d</sup>, Jens Baumert PhD <sup>d</sup>, Delilah Zabaneh PhD <sup>d</sup>, <sup>e</sup>, Jessica van Setten PhD <sup>d</sup>, Ivana Isgum PhD <sup>f</sup>, Tessell E. Galesloot PhD <sup>k</sup>, Johannes Arengård MD <sup>d</sup>, <sup>g</sup>, Philippe Amouyel MD, PhD <sup>d</sup>, Stela Trompett PhD <sup>d</sup>, <sup>h</sup>, Melanie Walderberger PhD, MPH <sup>d</sup>, <sup>i</sup>, Marcus Dörr MD <sup>d</sup>, <sup>j</sup>, Patrik K. Magnusson PhD <sup>d</sup>, Vilmarits Giedraitis PhD <sup>d</sup>, Anders Larsson MD, PhD <sup>d</sup>, <sup>j</sup>, Folkert W. Asselbergs MD, PhD <sup>d</sup>, <sup>k</sup>, <sup>l</sup>, <sup>m</sup>, <sup>n</sup>, <sup>o</sup>

 Show more

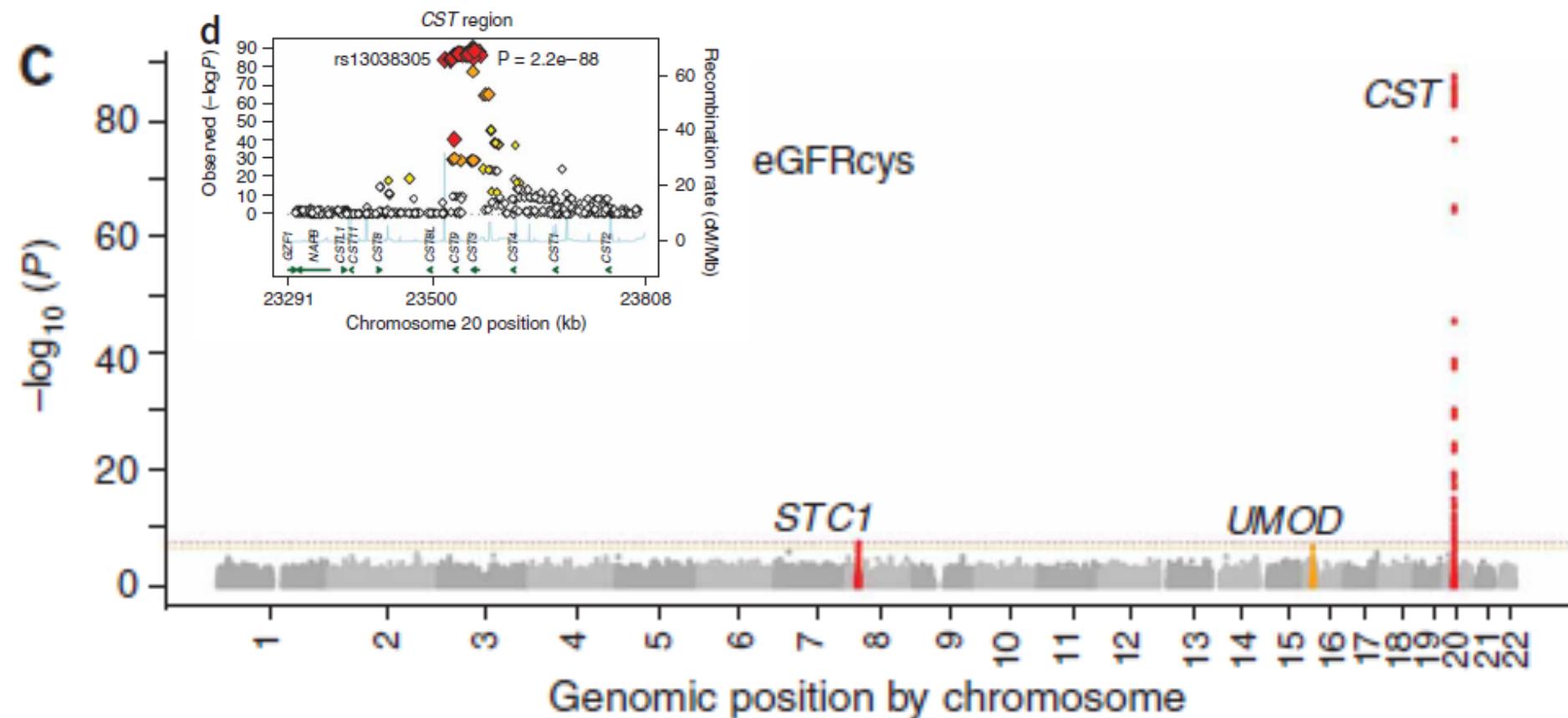
<https://doi.org/10.1016/j.jacc.2016.05.092>

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# GWAS: locus with *CST3* pops up (naturally)

- Four Genome-Wide Association Study Meta-Analyses of eGFR and CKD revealed a region containing *CST3* (Cystatin C) as significantly associated
  - Identification of three loci associated with eGFRcys aka CystC expression: *STC1*, *UMOD*, *CST3*



# Over 75,000 individuals included

**TABLE 1** Characteristics of Prospective Cohorts

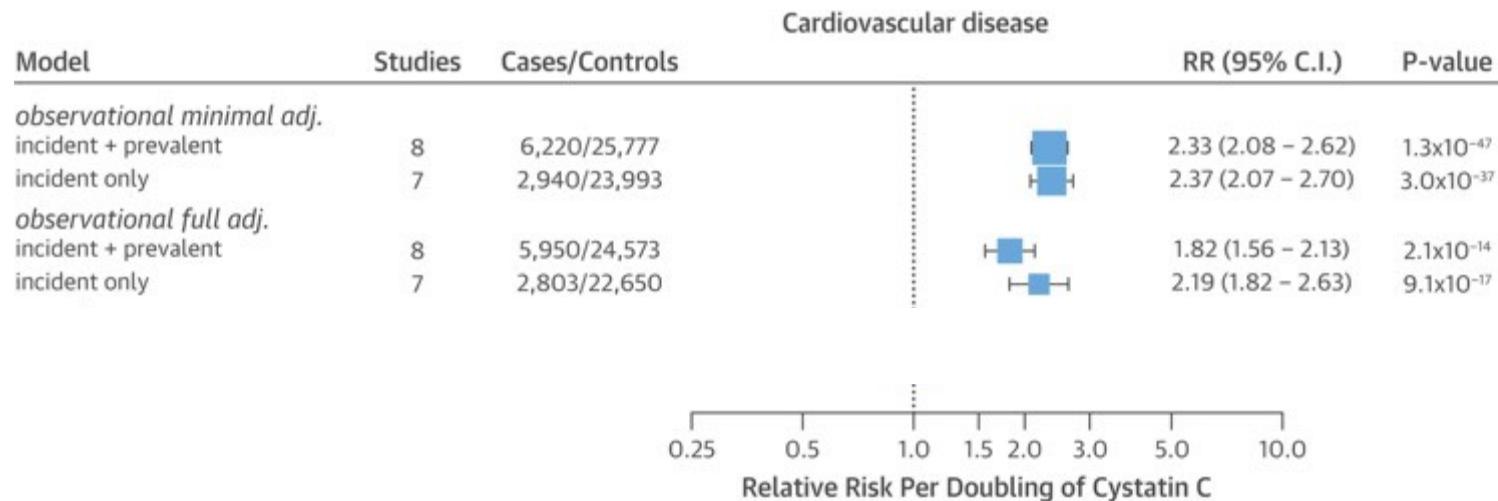
Study	Total	SNP*	Cystatin C†	CVD‡	CHD‡	IS‡	HF‡	MI‡	Male	Age (yrs)	Cystatin C (mg/dl)
3C	6,440	6,435	1,244	1,717	1,235	459	439	486	39.19	74.30 ± 5.52	0.92 ± 0.24
EPIC-NL	6,265	5,192	—	1,967	1,430	537	—	1,430	22.39	53.80 ± 10.23	—
GOSH	1,478	1,479	—	493	111	235	233	—	42.08	51.08 ± 11.86	—
HRS	7,844	5,585	5,777	—	—	—	—	—	—	—	0.64 ± 0.34
KORA	4,856	1,867	4,676	540	341	255	—	341	49.53	49.75 ± 14.11	0.80 ± 0.21
NBS	1,819	1,297	—	66	—	66	—	170	49.48	61.05 ± 10.26	—
PIVUS	1,016	949	1,004	255	175	71	75	105	49.90	70.20 ± 0.17	0.90 ± 0.19
PREVEND	3,245	3,245	3,245	236	190	58	—	—	50.26	49.42 ± 12.25	0.87 ± 0.17
PROSPER§	5,244	5,150	—	2,561	2,034	779	211	762	48.13	75.34 ± 3.35	—
Rotterdam	7,983	5,974	3,906	3,579	1,934	1,328	1,625	1,176	38.90	73.06 ± 7.49	1.11 ± 0.28
SHIP	3,224	3,224	3,212	114	19	87	—	134	48.08	54.46 ± 15.26	0.88 ± 0.30
Tromsø	6,129	—	6,129	1,251	—	494	—	881	47.59	60.59 ± 10.25	0.86 ± 0.18
TWINGENE	6,902	6,902	6,740	932	610	287	206	—	47.23	64.83 ± 8.26	1.02 ± 0.30
ULSAM	1,221	1,107	1,193	503	285	175	220	—	100.00	71.00 ± 0.64	1.25 ± 0.27
WHI	7,854	7,844	—	4,831	2,934	2,115	—	2,934	0.00	67.97 ± 6.58	—
Whitehall II	4,961	5,011	—	349	254	111	—	254	74.58	49.19 ± 5.99	—
Overall	76,481	61,261	37,126	19,394	11,552	7,057	3,009	8,673	—	—	—

Values are n, %, or mean ± SD. \*Total number of individuals with genotype data. †Genetic data were available in 29,805 of the 37,126 individuals that had values for cystatin C, which we used to associate rs911119 with circulating cystatin C. For the genetic analysis of CVD, CHD, IS, and HF, cohorts that contributed toward consortia were excluded.

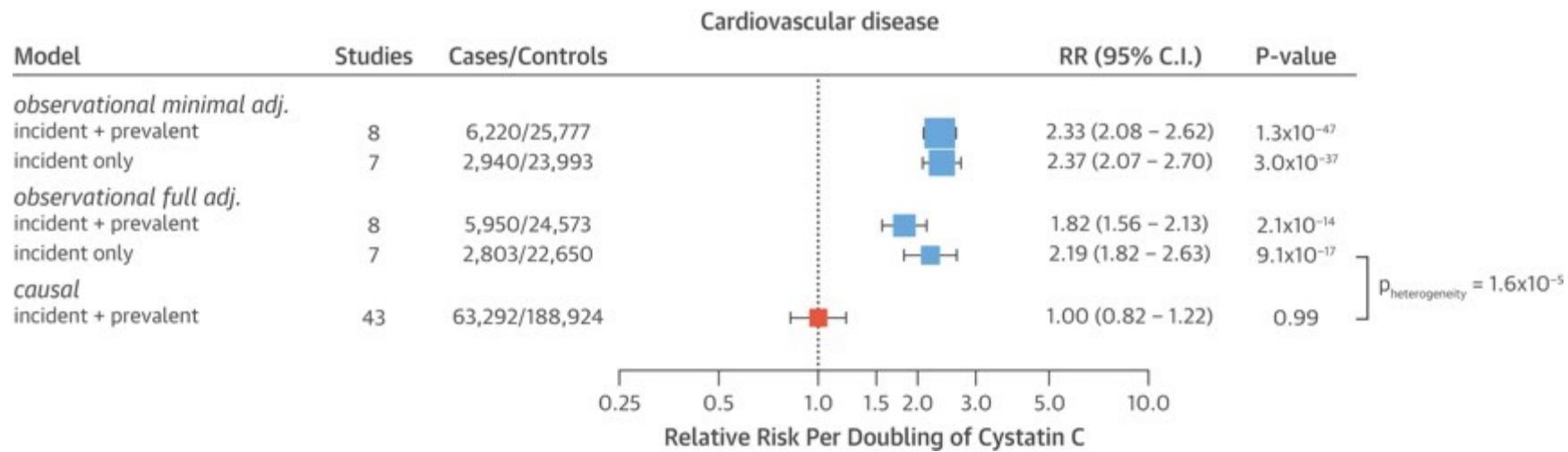
‡Indicates total incident and prevalent cases of disease or composite diseases in the case of CVD. §PROSPER is a randomized clinical trial. ||For the association of SNP with cystatin C concentrations, 9,488 samples were available in TWINGENE.

CHD = coronary heart disease; CVD = cardiovascular disease; HF = heart failure; IS = ischemic stroke; MI = myocardial infarction; SNP = single-nucleotide polymorphism.

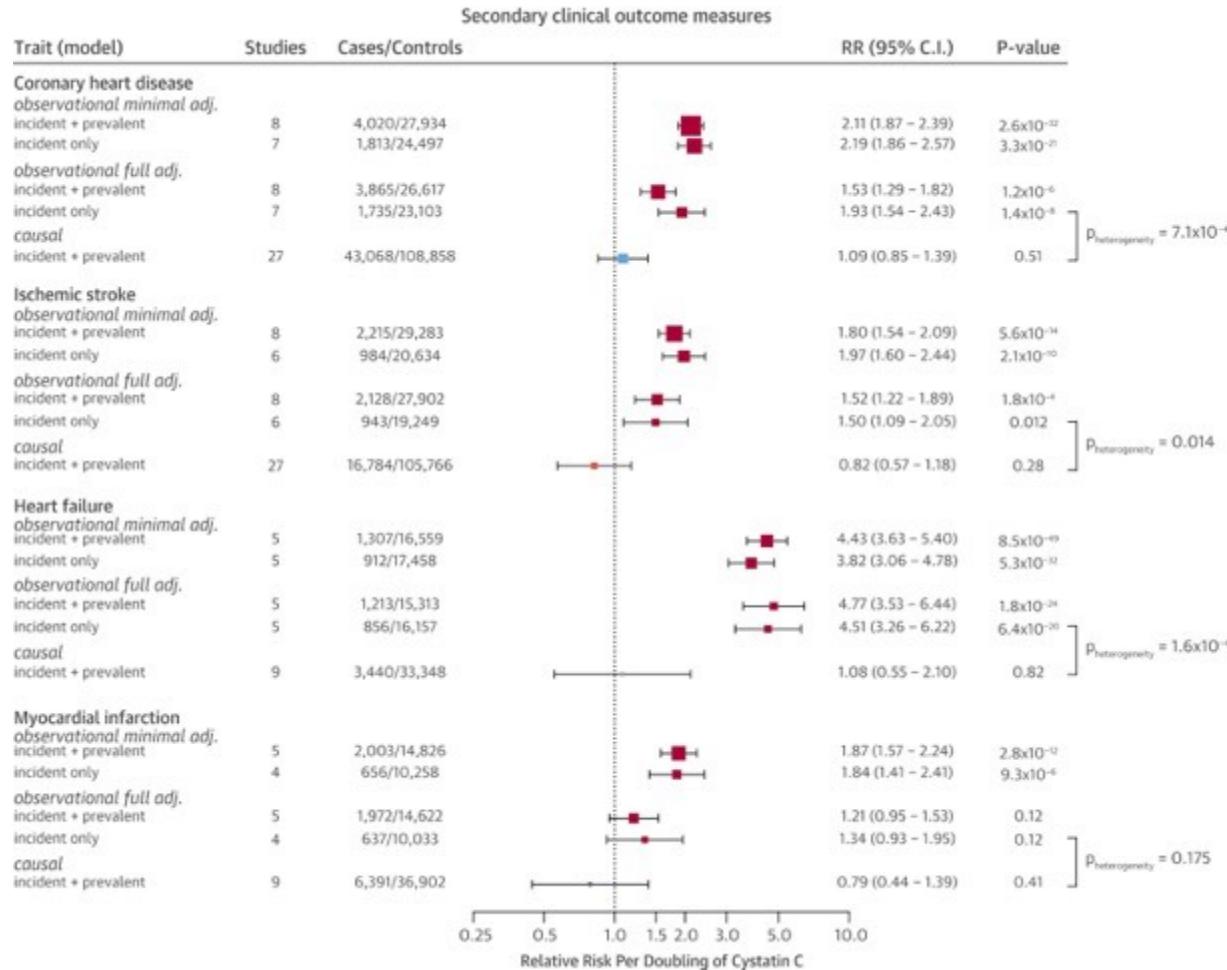
# Cystatin C associates to CVD in observational studies



# No causal effect of Cystatin C on CVD



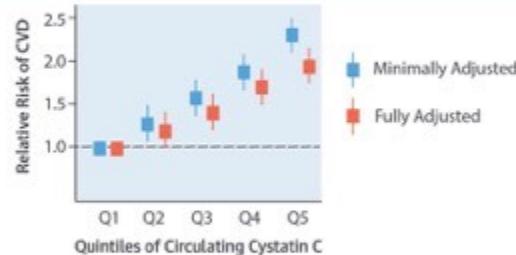
# Secondary clinical endpoints: nada, nothing, zip



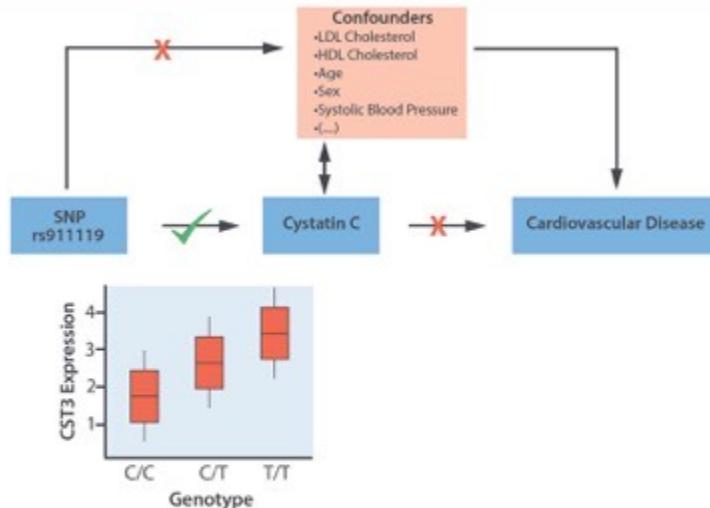
# Cystatin C levels are not causal to CVD risk

## CENTRAL ILLUSTRATION: Assessing Causality of Cystatin C in CVD

### A. Observational Epidemiology

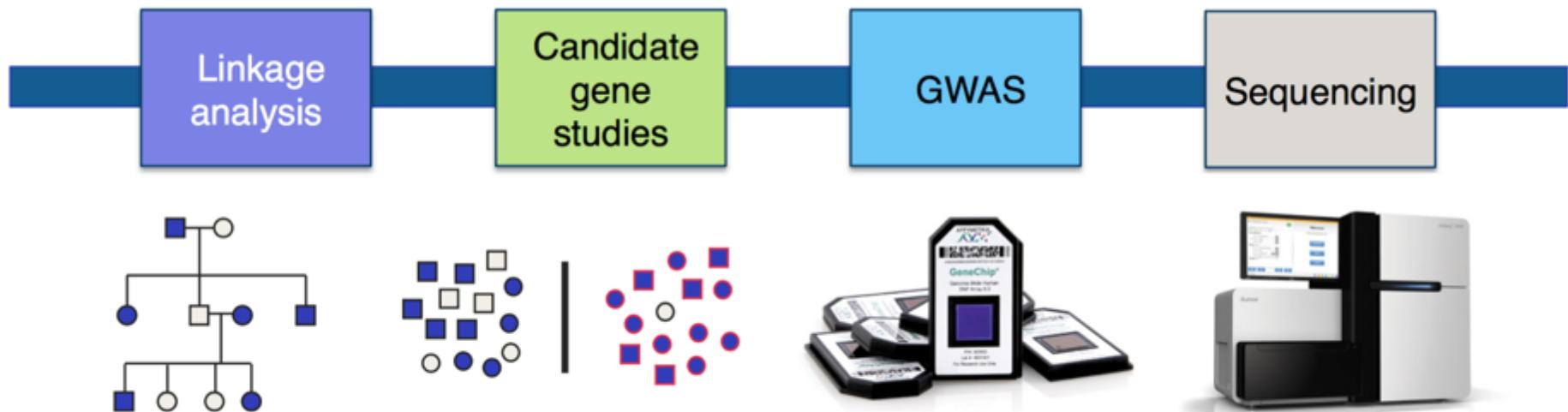


### B. Mendelian Randomization



van der Laan, S.W. et al. J Am Coll Cardiol. 2016;68(9):934-45.

# Drug discovery & validation



# Drug discovery

- Each locus likely harbors a disease-driving gene (or regulatory element)
- The magnitude of the odds ratio does not indicate
  - Potential biological value
  - Potential for therapy (“druggability”)
- Examples
  - *PPARG* in type 2 diabetes (thiazolidinediones)
  - *KCNJ11* and type 2 diabetes (sulfonylureas)
  - *PCSK9* and myocardial infarction (*PCSK9* inhibitors)

ORIGINAL ARTICLE

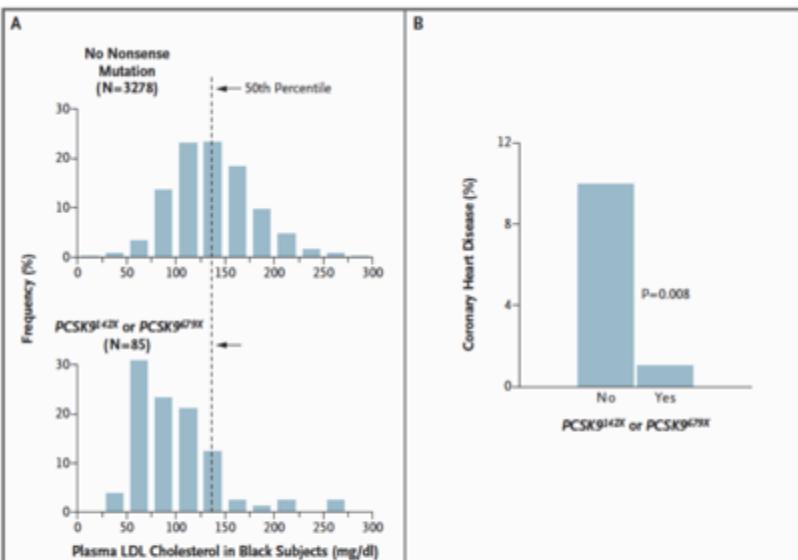
## Sequence Variations in PCSK9, Low LDL, and Protection against Coronary Heart Disease

Jonathan C. Cohen, Ph.D., Eric Boerwinkle, Ph.D., Thomas H. Mosley, Jr., Ph.D., and Helen H. Hobbs, M.D.

- **PCSK9 first discovered in familial hypercholesterolemia**
- Then discovered in a GWAS of EOMI

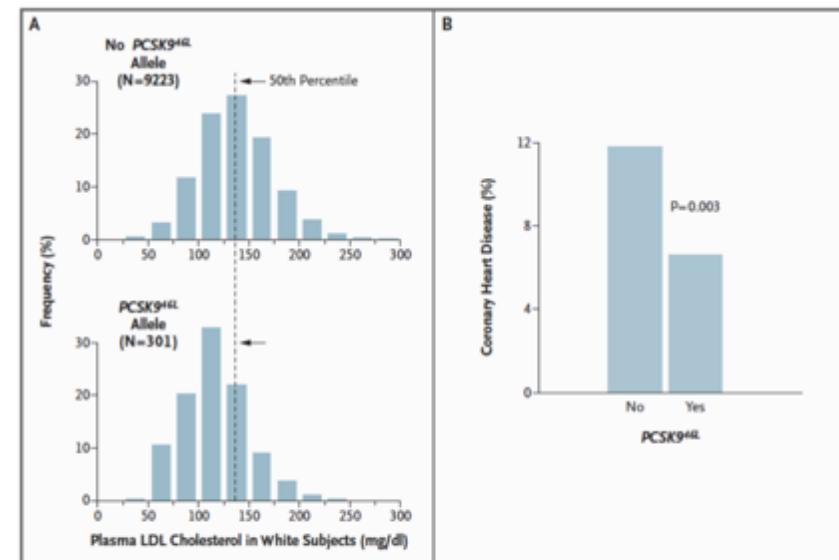


# LDL and PCSK9 in two populations



**Figure 1.** Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Heart Disease (Panel B) among Black Subjects, According to the Presence or Absence of a  $PCSK9^{142X}$  or  $PCSK9^{678X}$  Allele.

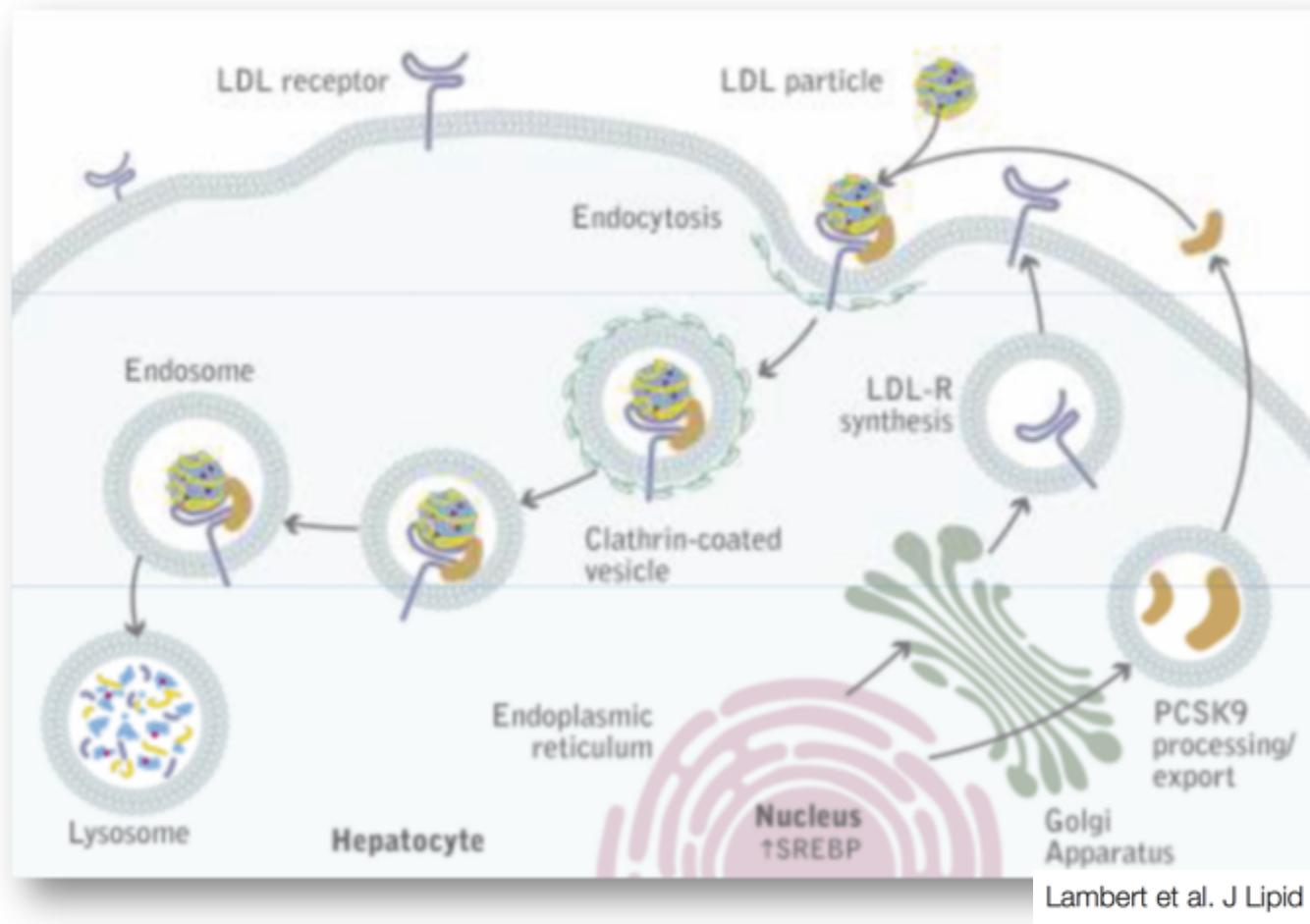
In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 3278 black subjects who did not have a  $PCSK9^{142X}$  or  $PCSK9^{678X}$  allele (top) is compared with the distribution of levels among the 85 black subjects who had one of these two alleles (bottom). Panel B shows the percentage of participants from these two groups who had no evidence of coronary heart disease at baseline and in whom coronary heart disease developed during the 15-year follow-up period. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.



**Figure 2.** Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Events (Panel B) among White Subjects, According to the Presence or Absence of a  $PCSK9^{46L}$  Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 9223 white subjects who did not have a  $PCSK9^{46L}$  allele (top) is compared with the distribution of levels among the 301 white subjects who were either heterozygous or homozygous for this allele (bottom). Panel B shows the percentage of participants from these two groups who had no evidence of coronary heart disease at baseline and in whom coronary heart disease developed during the 15-year follow-up period. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

# PCSK9 promotes *LDLR* degradation



Lambert et al. J Lipid Res 2012

# Conclusions

- The genomic sequence is set at conception, no need to worry about confounding factors
- GWAS have been and will continue to be very successful
- Still need large sample sizes for sufficient power
- Complex genetics studies useful to gain information causality and drug discovery
- The jury is still out with respect to risk prediction
- Interpretation and translation will be the major challenge in the next decade

# Cardiovascular Genetic Research

## Laboratory of Clinical Chemistry and Hematology

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### Cardiovascular Genetics

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### Research topics S.W. van der Laan

Biomarker Discovery & Validation

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Cardiovascular Genomics

