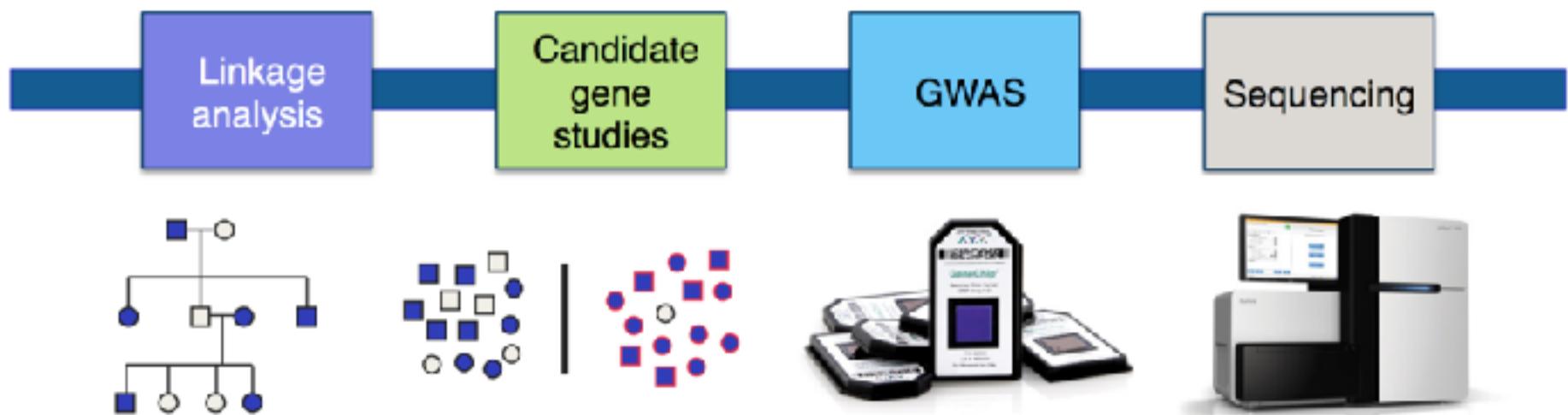
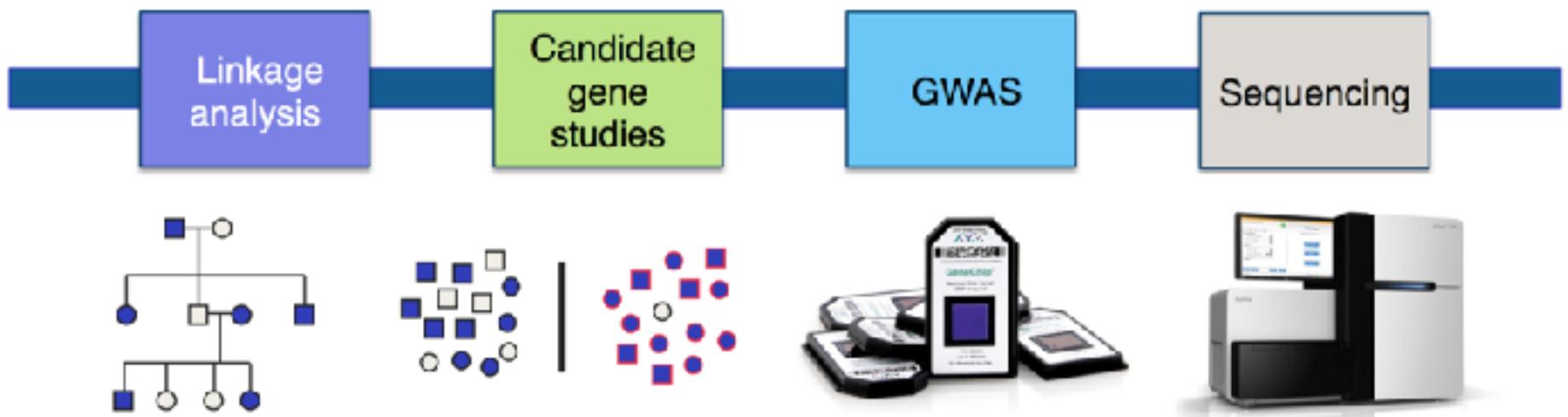


What have we learned in the field of cardiovascular genetics?



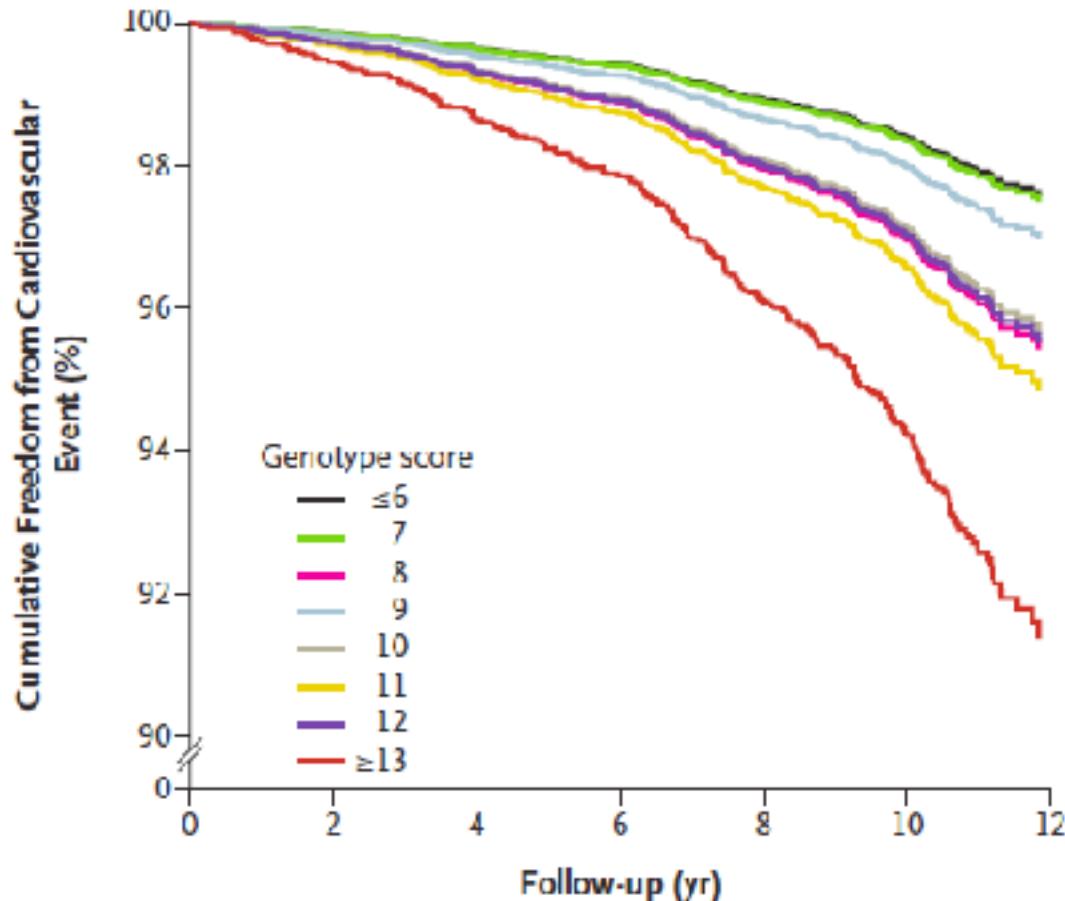
Prediction?



9 SNPs associations with CVD

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

	Multivariable-Adjusted Hazard Ratio (95% CI)	P Value
Age, per SD	1.37 (1.37–2.07)	<0.001
Male sex	1.61 (1.29–2.17)	0.007
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
Hypertension index, per SD	1.04 (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 (0.99–1.38)	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

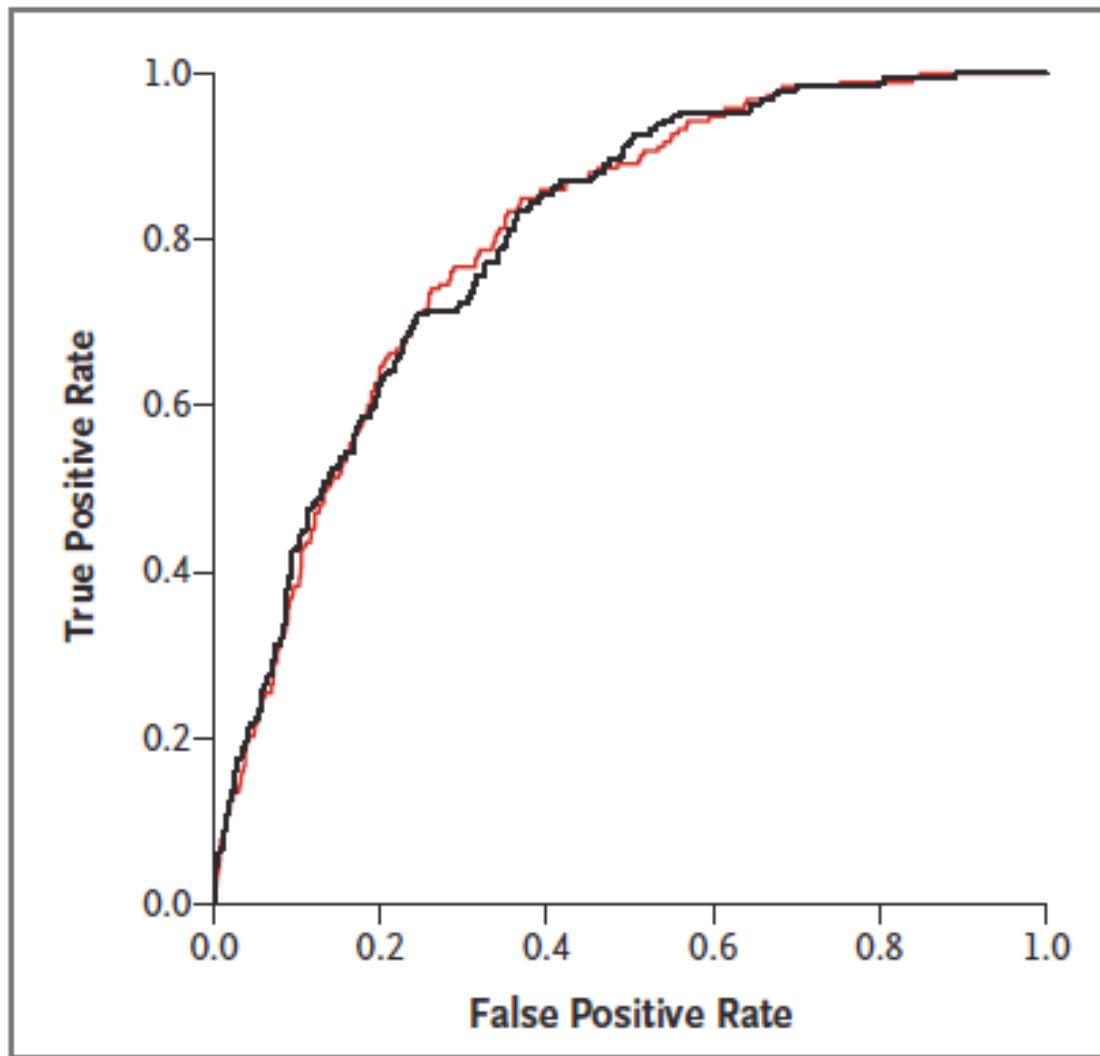


Figure 2. Receiver-Operating-Characteristic (ROC) Curves for Incident Myocardial Infarction, Ischemic Stroke, or Death from Coronary Heart Disease during 10-Year Follow-up.

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 ▾

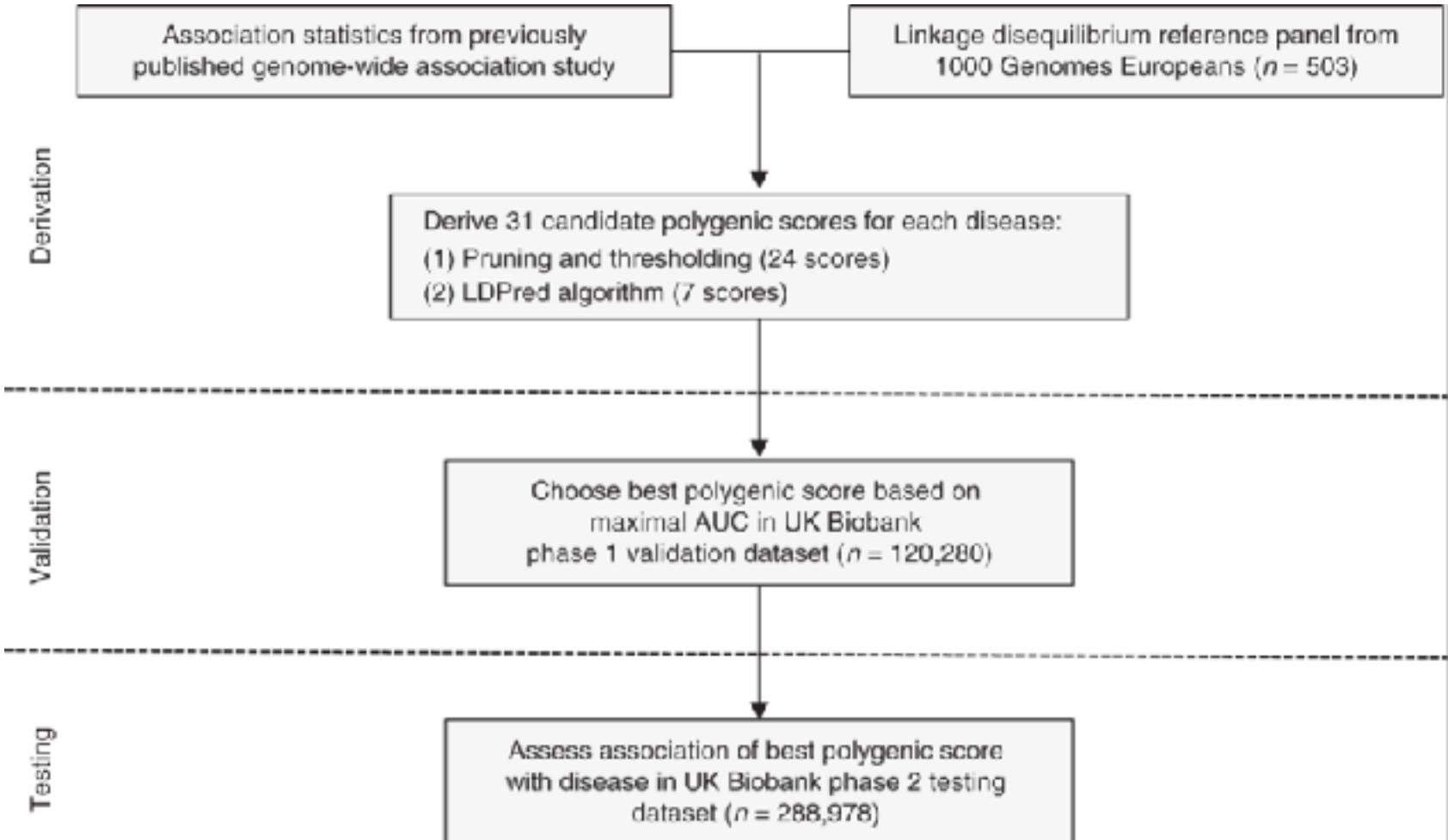
Letter | Published: 13 August 2018

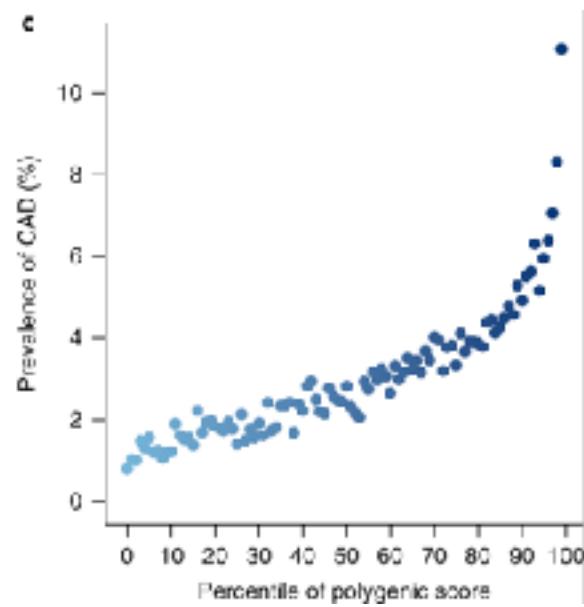
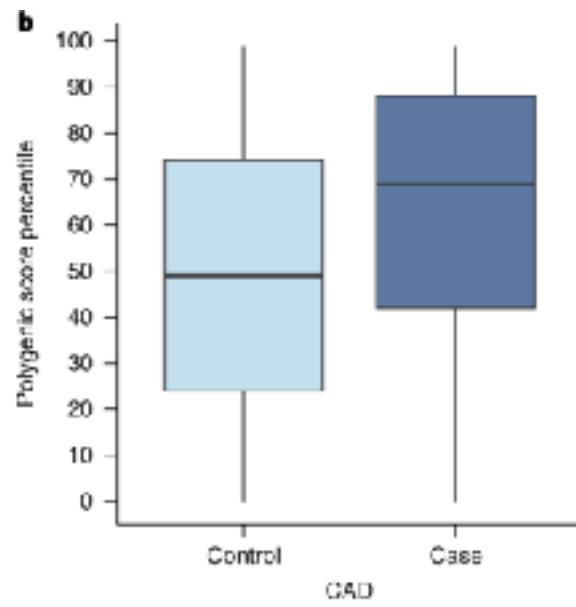
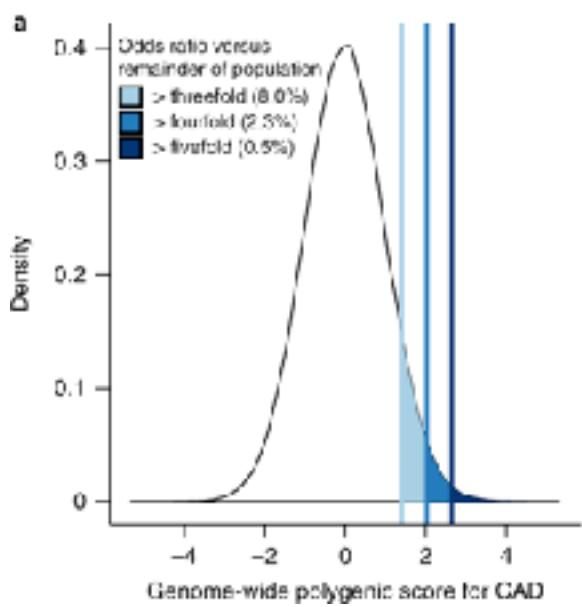
Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations

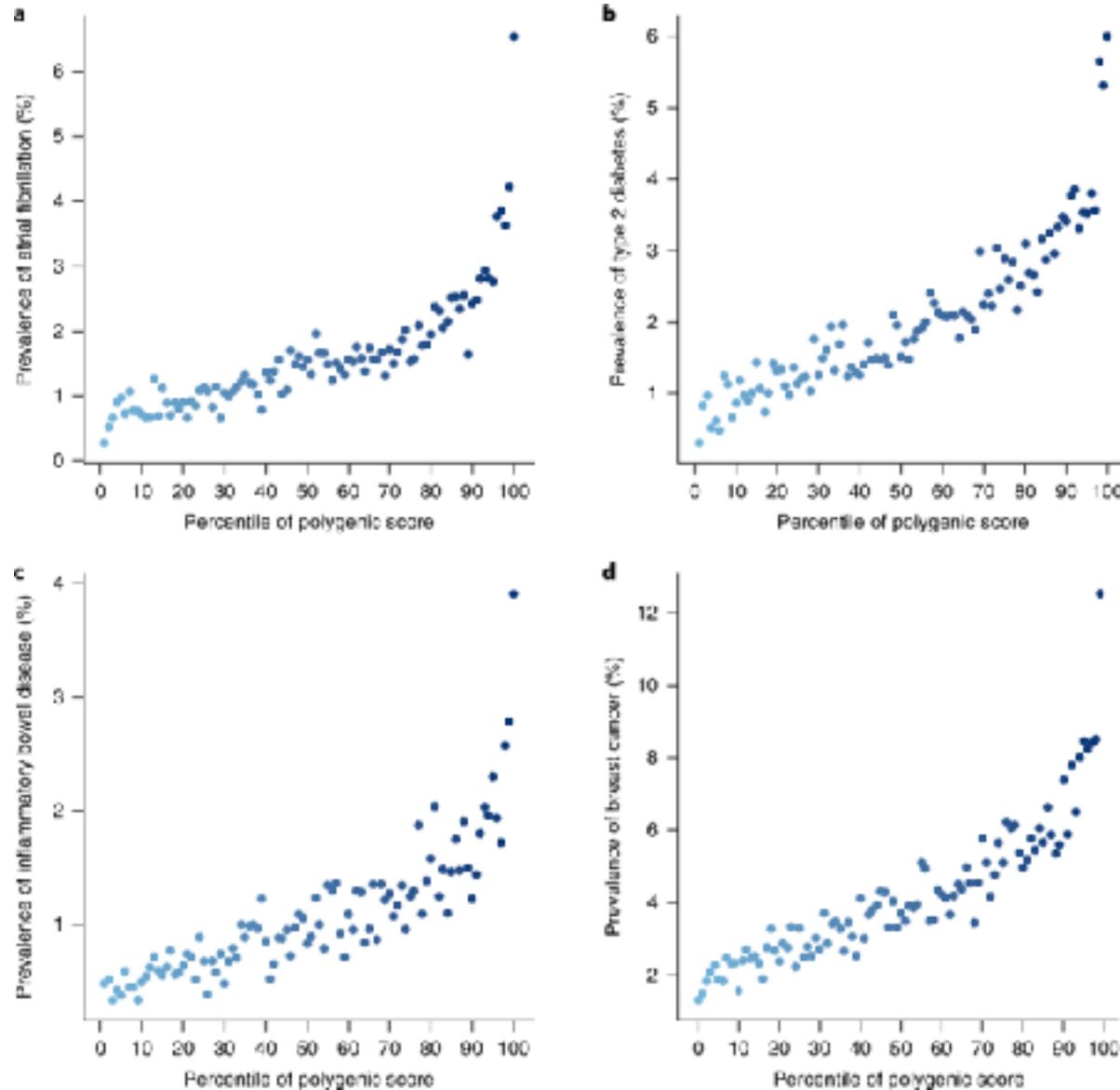
Amit V. Khera, Mark Chaffin, Krishna G. Aragam, Mary E. Haas, Carolina Roselli, Seung Hoan Choi, Pradeep Natarajan, Eric S. Lander, Steven A. Lubitz, Patrick T. Ellinor & Sekar Kathiresan✉

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

Include millions of variants of small effects







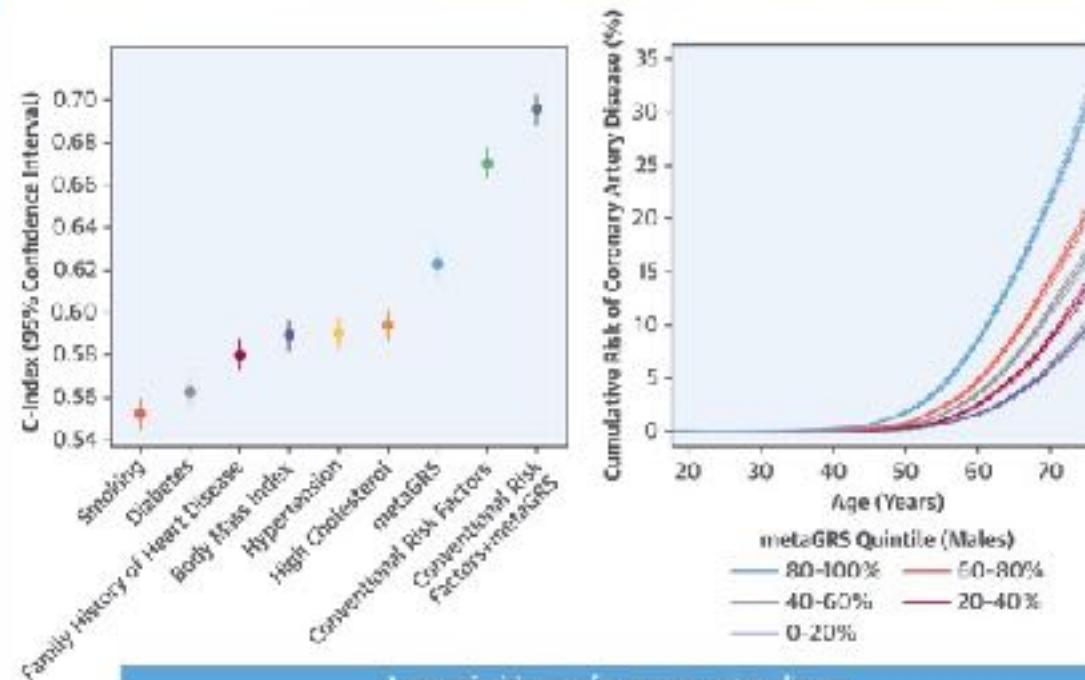
High GPS definition	Individuals in testing dataset (n)	% of individuals
Odds ratio ≥3.0		
CAD	23,119/288,978	8.0
Atrial fibrillation	17,627/288,978	6.1
Type 2 diabetes	10,099/288,978	3.5
Inflammatory bowel disease	9,209/288,978	3.2
Breast cancer	2,369/157,895	1.5
Any of the five diseases	57,115/288,978	19.8
Odds ratio ≥4.0		
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
Odds ratio ≥5.0		
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 980,000 Adults

Implications for Primary Prevention

Michael Inouye, Ron Abramson, Christopher P. Nelson, Angela M. Wood, Michael J. Sweeney, Francis Dabholkar, Florence Y. Lai, Stephen Raptoge, Maria Brzozowska, Tingting Wang, Shu Yu, Thomas R. Field, Martin K. Reffert, Joanna Tsoukatos, Riaz S. Patel, Ruth J.F. Liss, Bernard Herveier, Harry Hershberger, John Thompson, Hugh Watkins, Panos Deloukas, Evangelia Tsilangkotimou, Adam S. Kermani, John Janeski, Nilesen J. Samani and for the UK Biobank CardioMetabolic Consortium CHD Working Group

CENTRAL ILLUSTRATION: Genomic Risk Score for Coronary Artery Disease

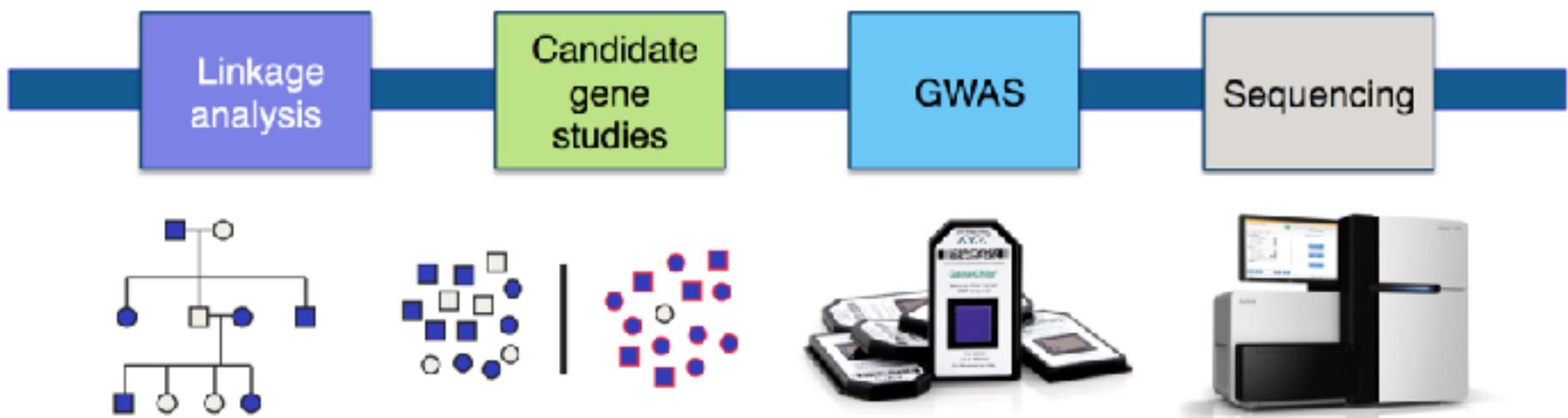


A genomic risk score for coronary artery disease

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.

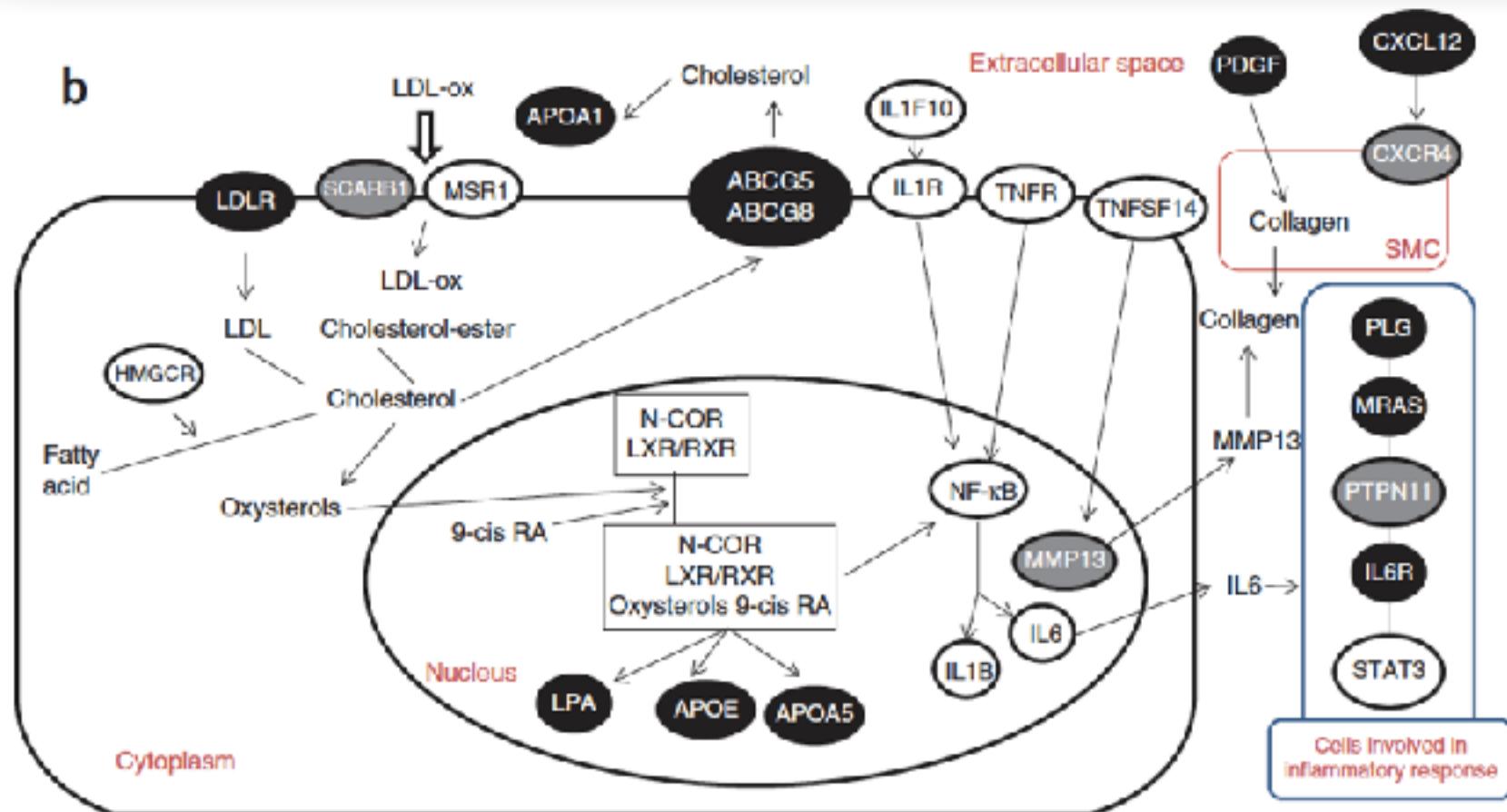
Biological mechanisms?



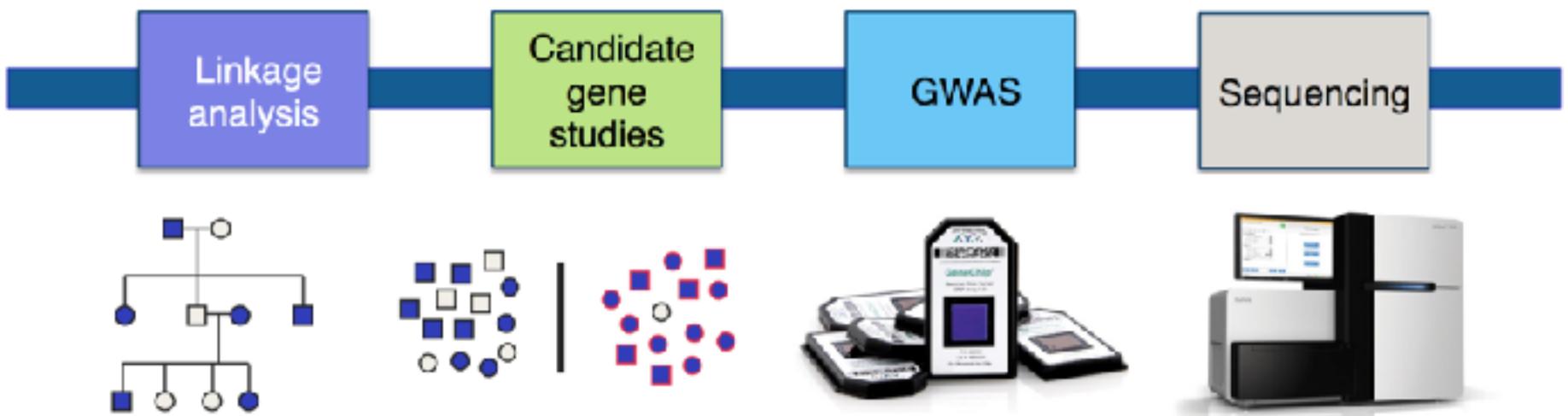
Large-scale association analysis identifies new risk loci for coronary artery disease

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

The CARDIoGRAMplusC4D Consortium¹



Causality: Mendelian Randomization?



APOLIPROTEIN E ISOFORMS, SERUM CHOLESTEROL, AND CANCER

SR.—It is unclear whether the relation between low serum cholesterol levels and cancer¹ 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.^{2,3} 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.³ Thus, naturally low cholesterol levels are sometimes associated with increased cancer risk.^{1,3}

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.⁴ 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%.⁵ 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,^{6,8} 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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1. McMichael AJ, Jensen OM, Parkin DM, Zardini DG. Dietary and endogenous cholesterol and human cancer. *Sympozial 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.
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4. Brown MS, Kovacs PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. *Science* 1981; 212: 628-35.
5. Utermann G, Steinmann B, Weber W. Genetic control of human apolipoprotein E polymorphism: comparison of one- and two-dimensional techniques of isoelectric analysis. *Hum Genet* 1982; 60: 944-51.
6. Utermann G, Kindermann I, Kaffarnik H, Siemers A. Apolipoprotein E phenotypes and hyperlipidemia. *Hum Genet* 1984; 65: 332-36.
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8. Utermann G. Genetic polymorphisms 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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IC focusing of plasma lipoproteins; epidemiologists interested in carcinogenesis and cancer should include it in their studies.

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MARTIJN B. KATAN

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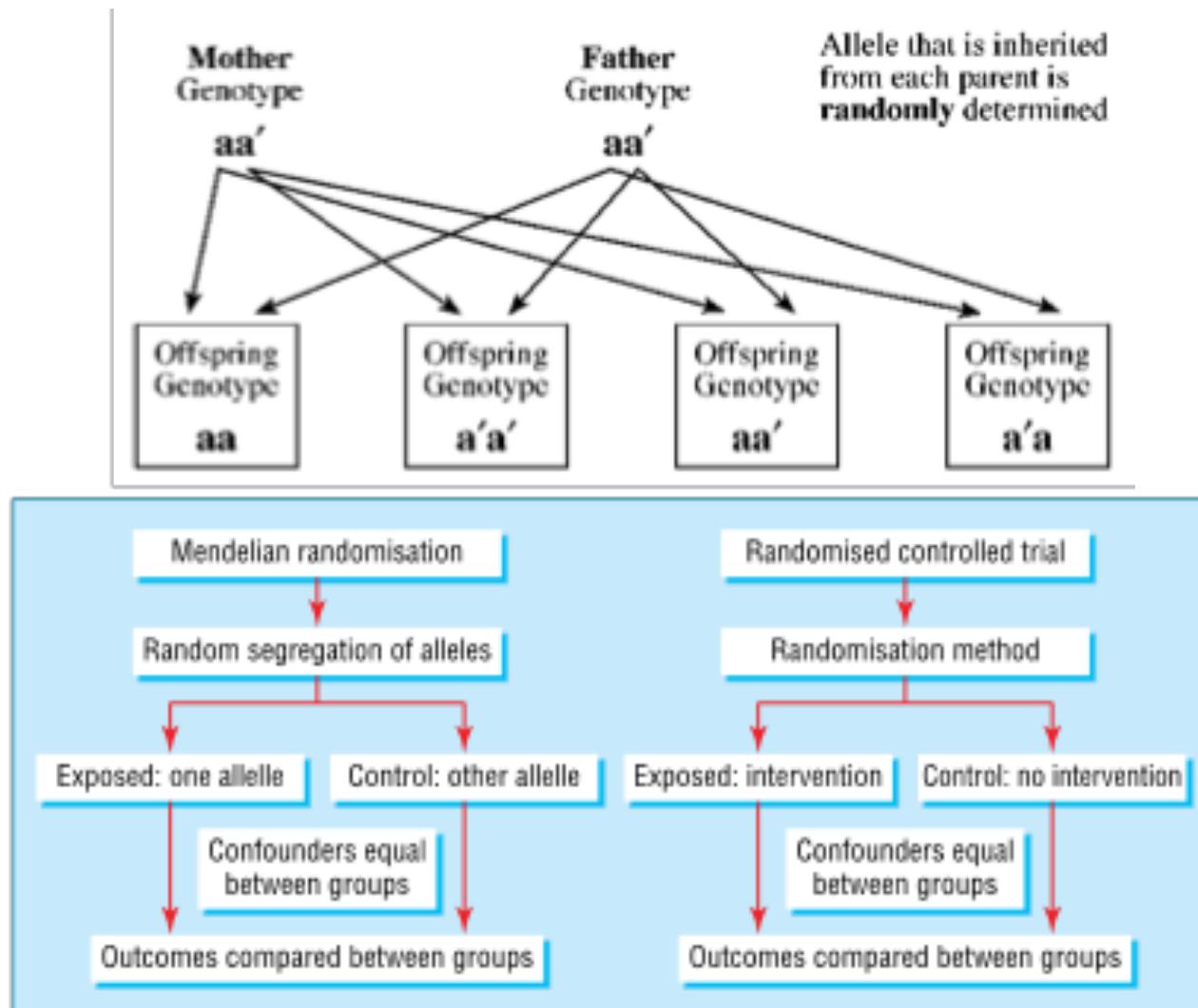
cardiovascular diseases. In: Nestel PJ, et al., eds. *Atherosclerosis VII: Proceedings of the Seventh International Atherosclerosis Symposium*. Amsterdam: Elsevier, 1986.

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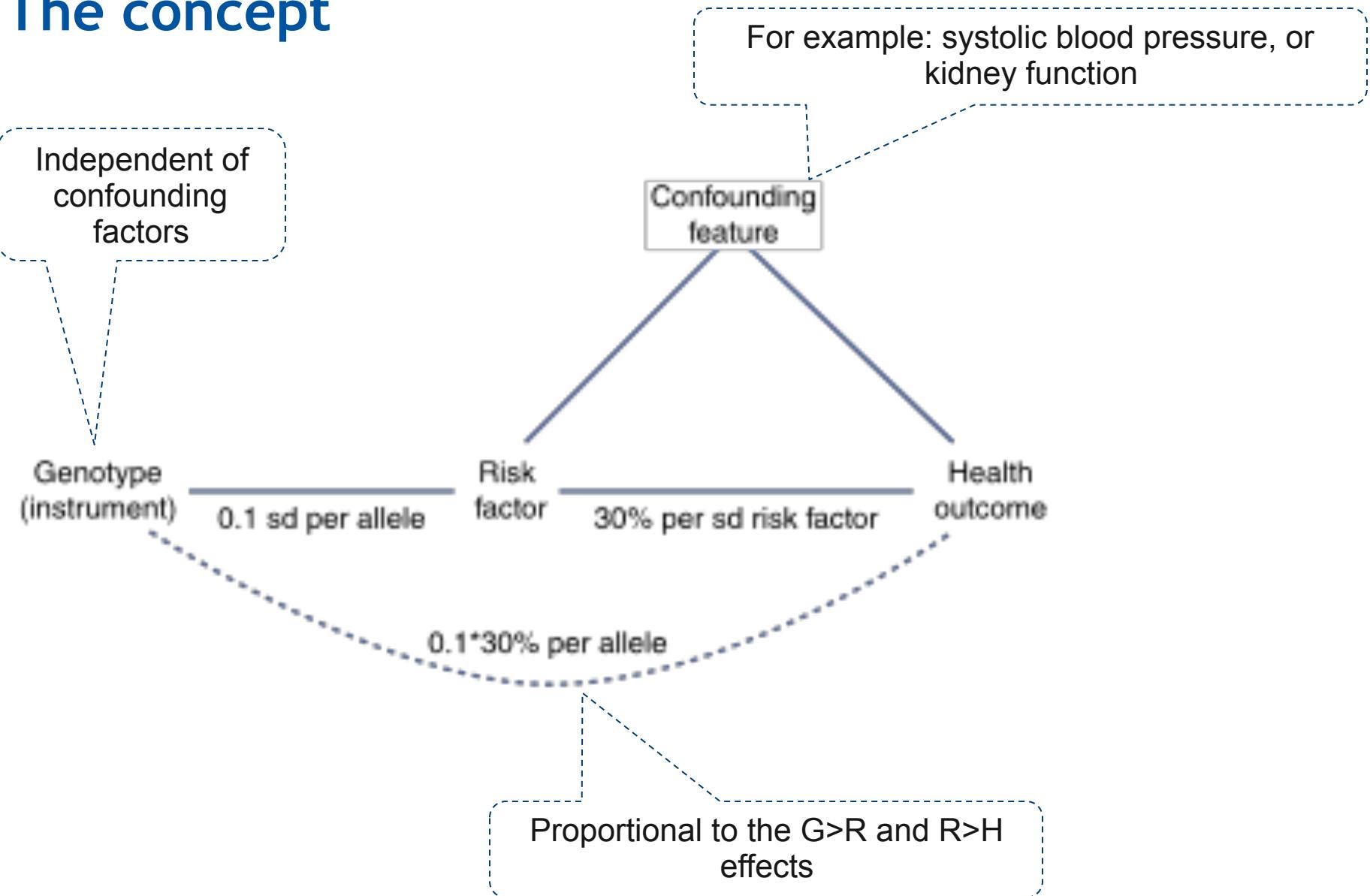


Gregor Johann Mendel - 20 July 1822 – 6 January 1884

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



The concept



Original Investigation

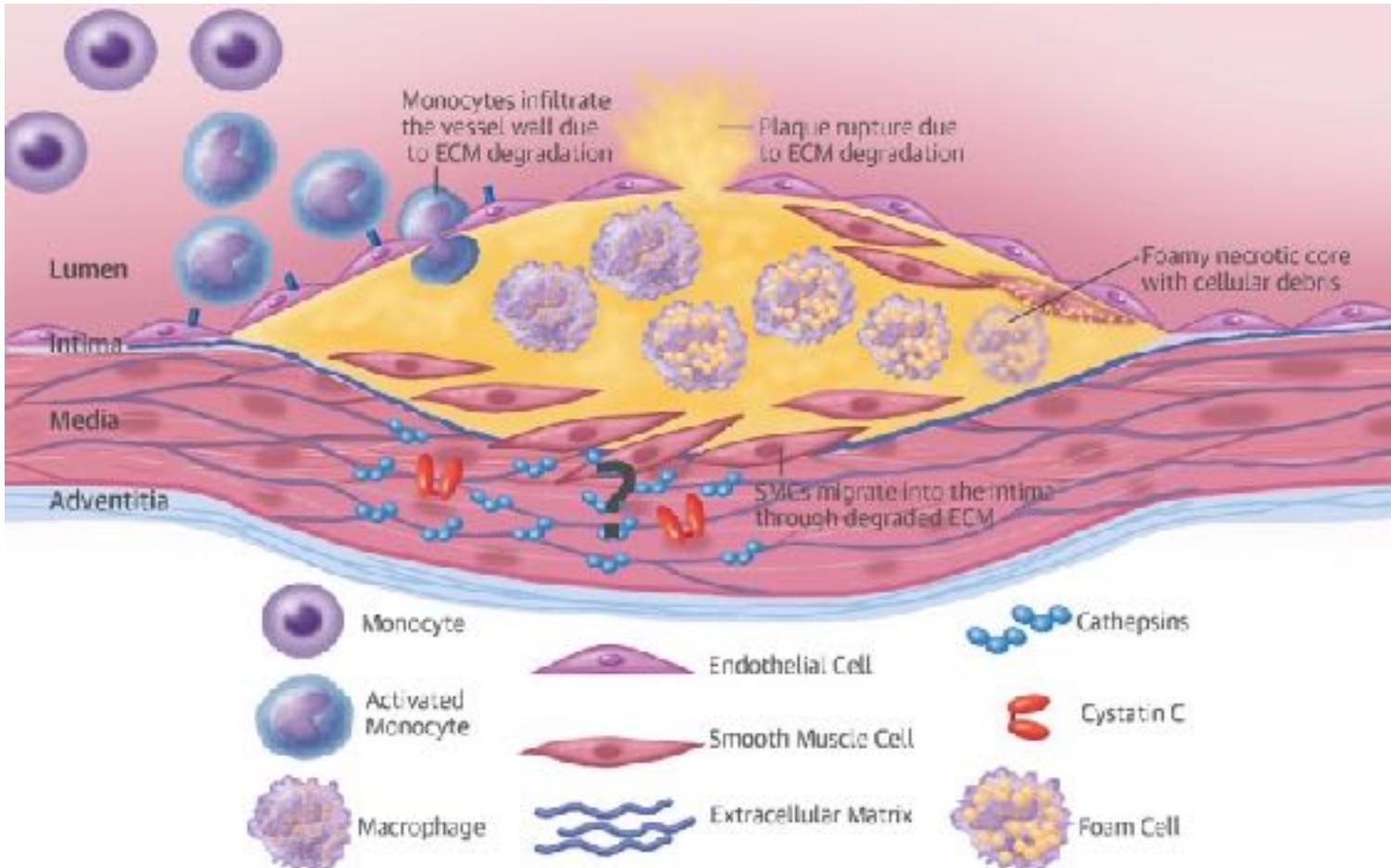
Cystatin C and Cardiovascular Disease: A Mendelian Randomization Study

Xander W. van der Velde MD, PhD,¹ Ines van der Heijden MD, PhD,¹ Jolanta Zelenaia PhD,¹ Joost den Heijer PhD,¹ Gertie Begeerse MSc,¹ Joris De Geer PhD,¹ Delphine Zabeau PhD,^{1,2} Jessica van Golen PhD,¹ Ines Rijntjes PhD,¹ Inez van Damme PhD,¹ Joanne Arengius PhD,¹ Priscilla van der Velde PhD,¹ Sander Trompett PhD,^{1,3} Valerie Walenberger PhD, MPH,^{1,4} Natasja den Dijck MD,^{1,5} Petrik L. Magnusson PhD,¹ Vilma van Oijen PhD,¹ Antje Lauten MD, PhD,¹ ... , Fulco P. Asebergen MD, PhD,^{1,6,7,8,9} and A. E.

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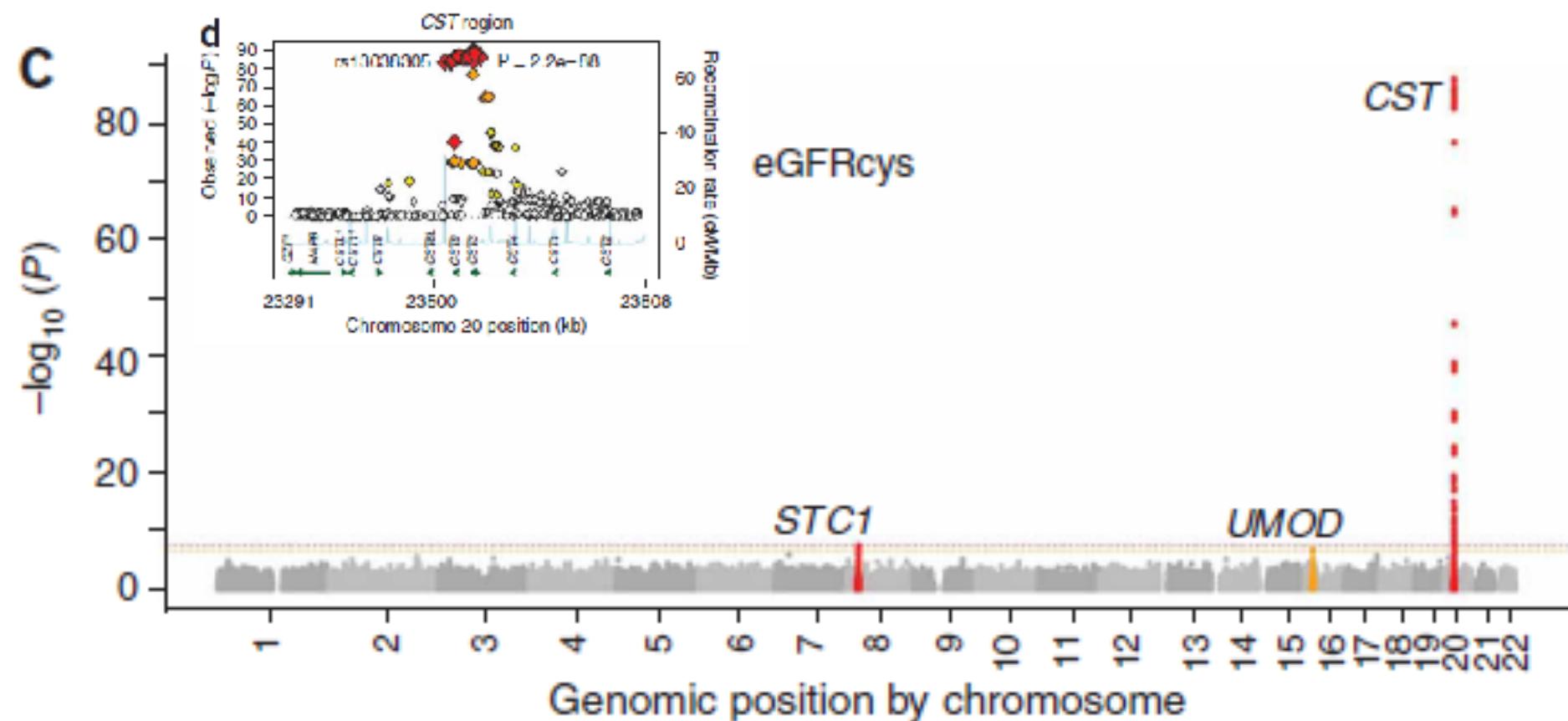
<https://doi.org/10.1016/j.jacc.2019.05.002>

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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

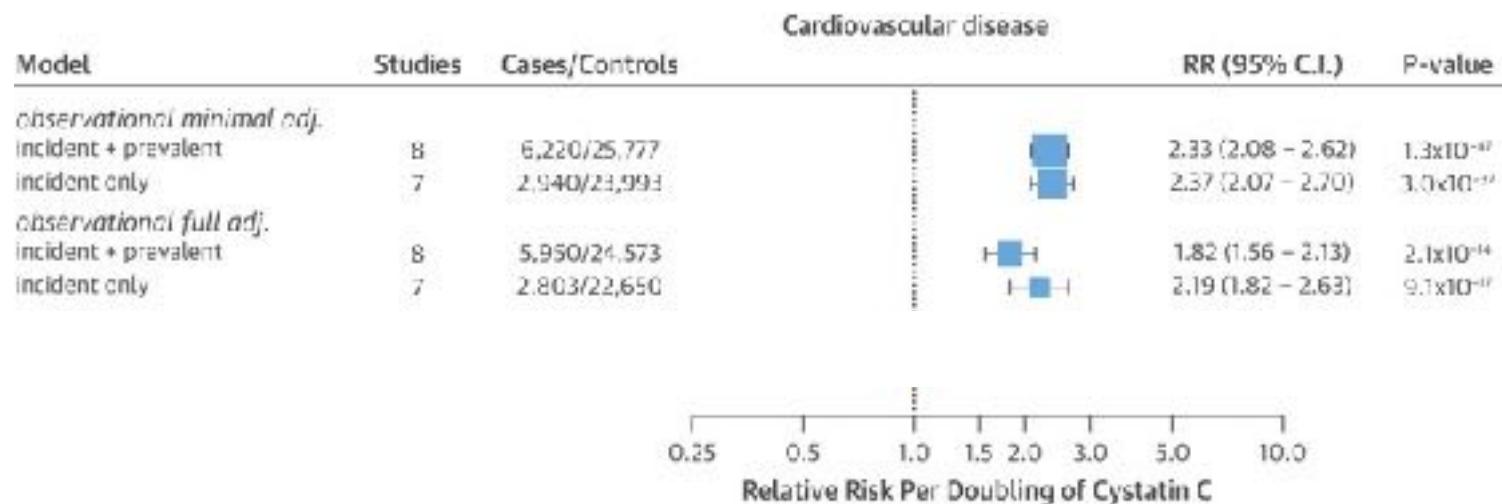
Study	Total	SNP*	Cystatin C†	CVD‡	CHD‡	IS‡	HF‡	MI‡	Male	Age (yrs)	Cystatin C (mg/dl)
3C	6,440	6,435	1,244	1,717	1,235	459	439	486	39.19	74.30 ± 5.52	0.92 ± 0.24
EPIC-NL	6,265	5,192	—	1,967	1,430	537	—	1,430	22.39	53.80 ± 10.23	—
GOSH	1,478	1,479	—	493	111	235	233	—	42.08	51.08 ± 11.86	—
HRS	7,844	5,585	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.

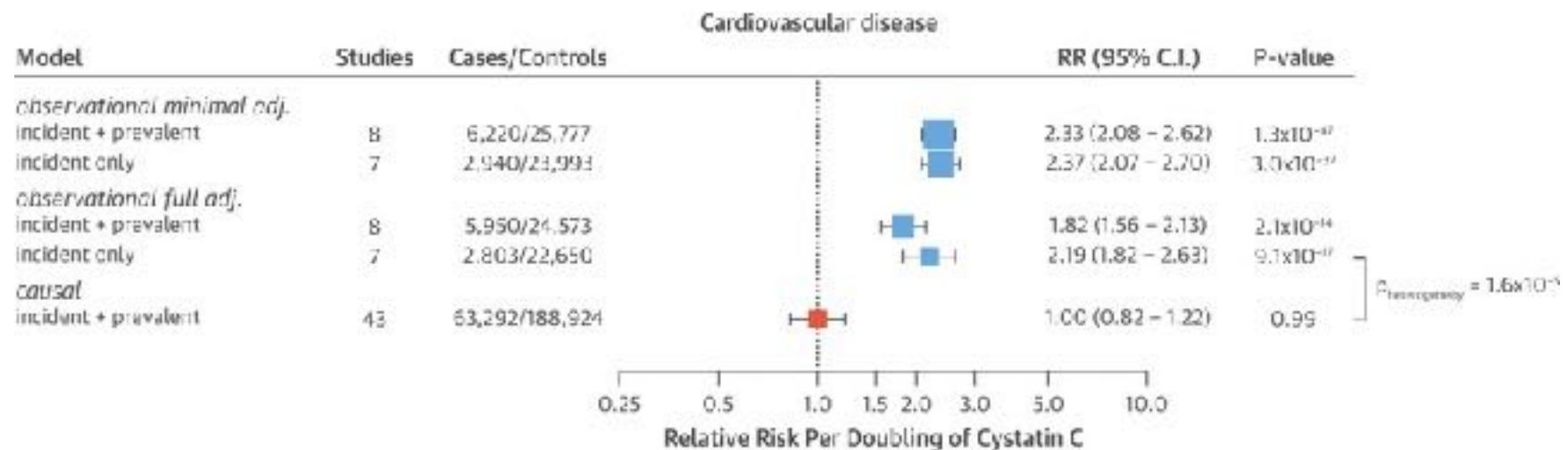
‡Indicates total incident and prevalent cases of disease or composite diseases in the case of CVD. §PROSPER is a randomized clinical trial. ||For the association of SNP with cystatin C concentrations, 9,488 samples were available in TWINGENE.

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

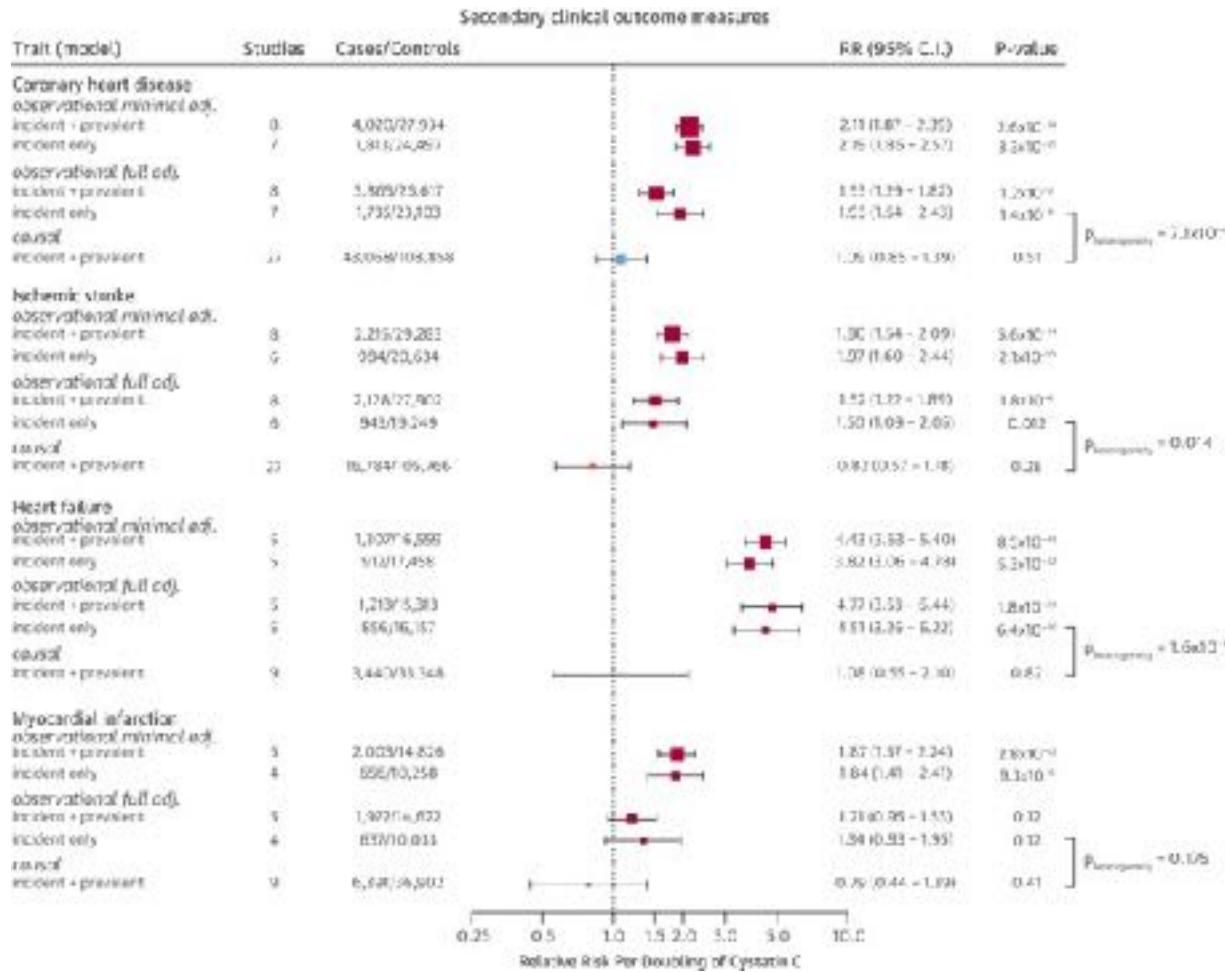
Cystatin C associates to CVD in observational studies



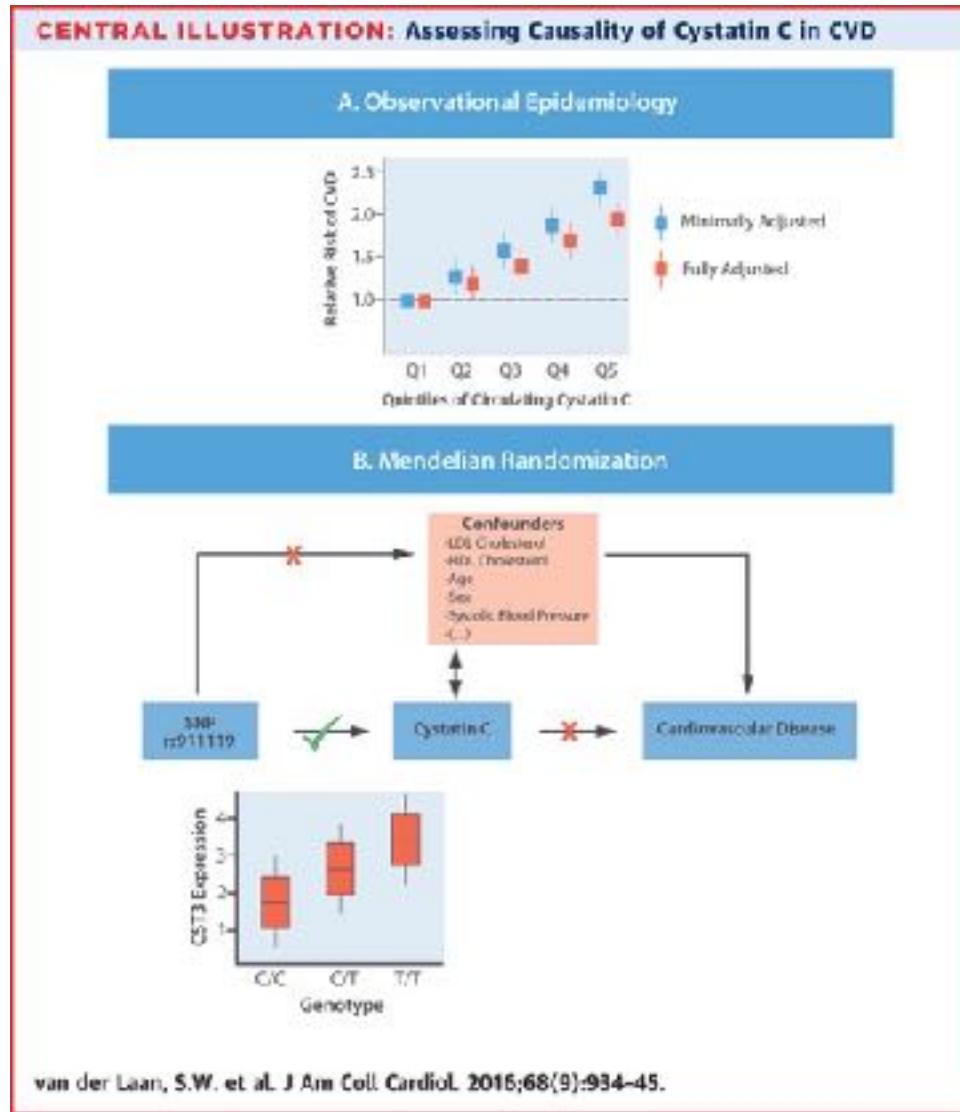
No causal effect of Cystatin C on CVD



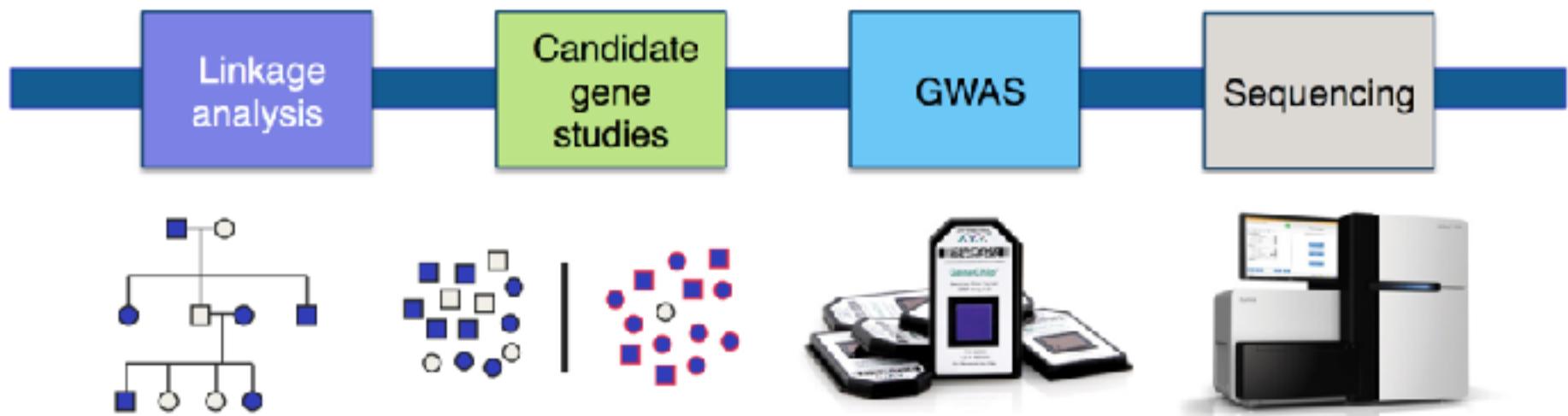
Secondary clinical endpoints: nada, nothing, zip



Cystatin C levels are not causal to CVD risk



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 Boenwinkle, 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

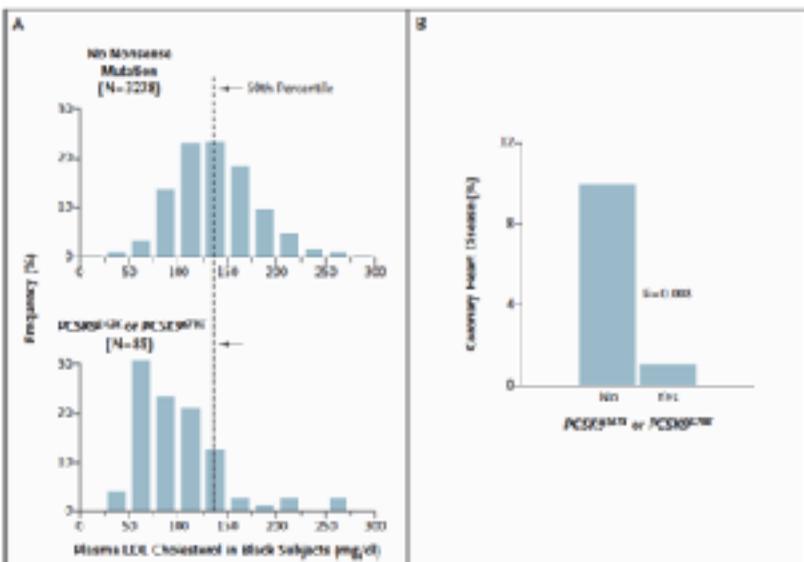


Figure 1. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Heart Disease (Panel B) among Black Subjects, According to the Presence or Absence of a *PCSK9^{L/F}* or *PCSK9^{H/H}* Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 328 black subjects who did not have a *PCSK9^{H/H}* or *PCSK9^{L/F}* allele (top) is compared with the distribution of levels among the 45 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.

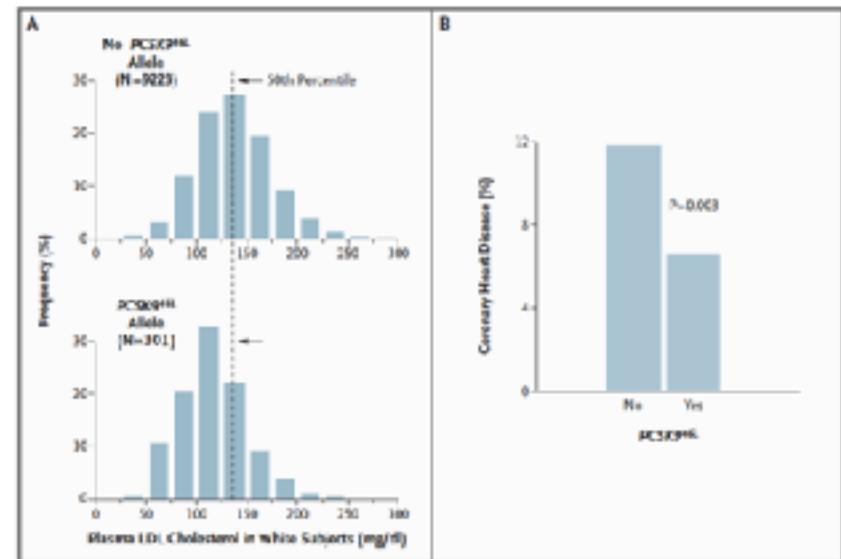
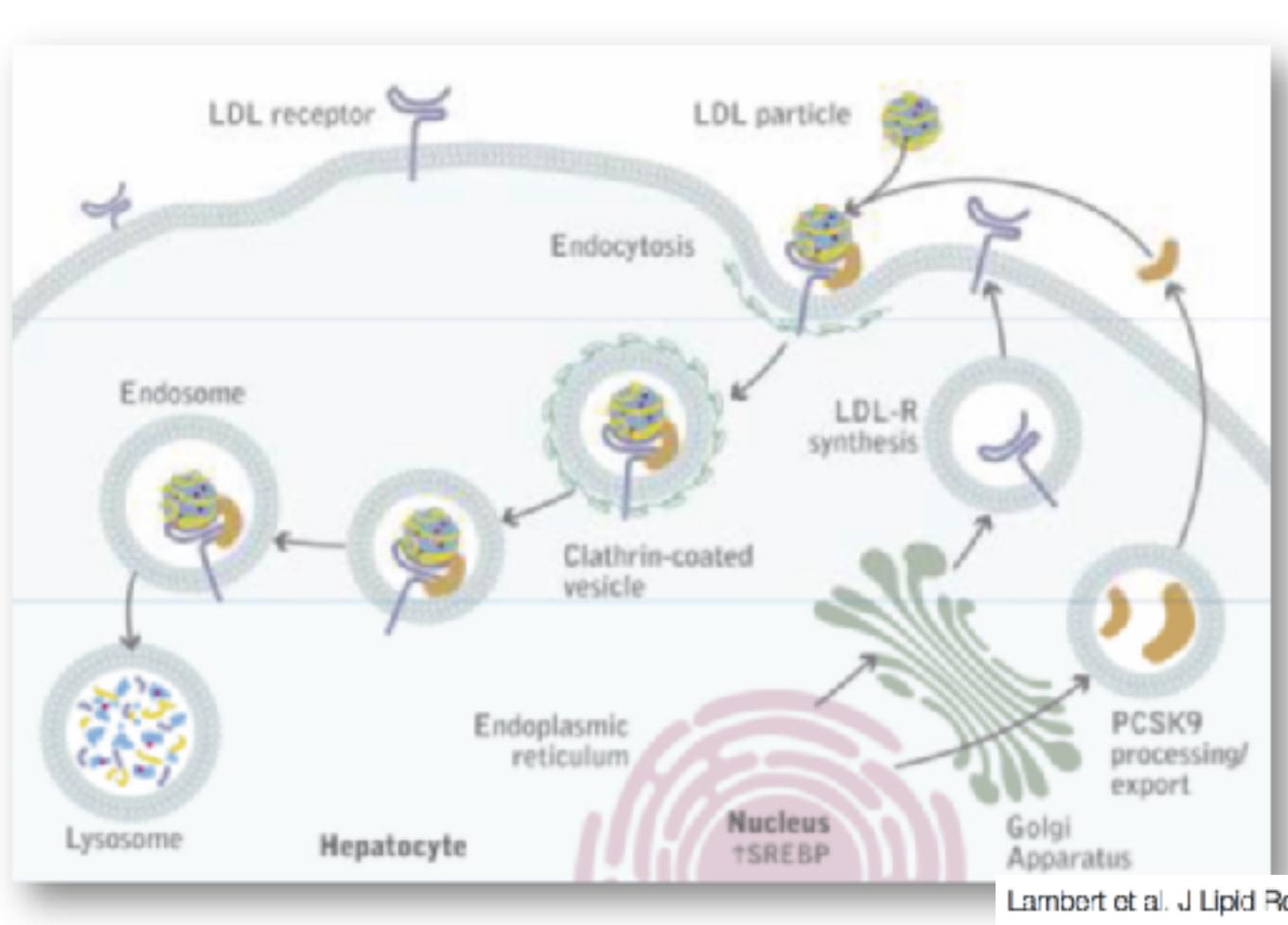


Figure 2. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Events (Panel B) among White Subjects, According to the Presence or Absence of a *PCSK9^{H/H}* Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 923 white subjects who did not have a *PCSK9^{H/H}* 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

Dr. Sander W. van der Laan

Cardiovascular Genetics

Prof. Dr. Folkert W. Asselbergs

Prof. Dr. Gerard Pasterkamp

Dr. Jessica van Setten

Dr. Magdalena Harakalova

Dr. Floriaan Schmidt

Dr. Michal Mokry

Research topics S.W. van der Laan

Biomarker Discovery & Validation

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Athero-Express | AAA-Express | CTMM | many

more



Cardiovascular Genomics

