

A primer in complex human genetics

Focused cardiovascular disease

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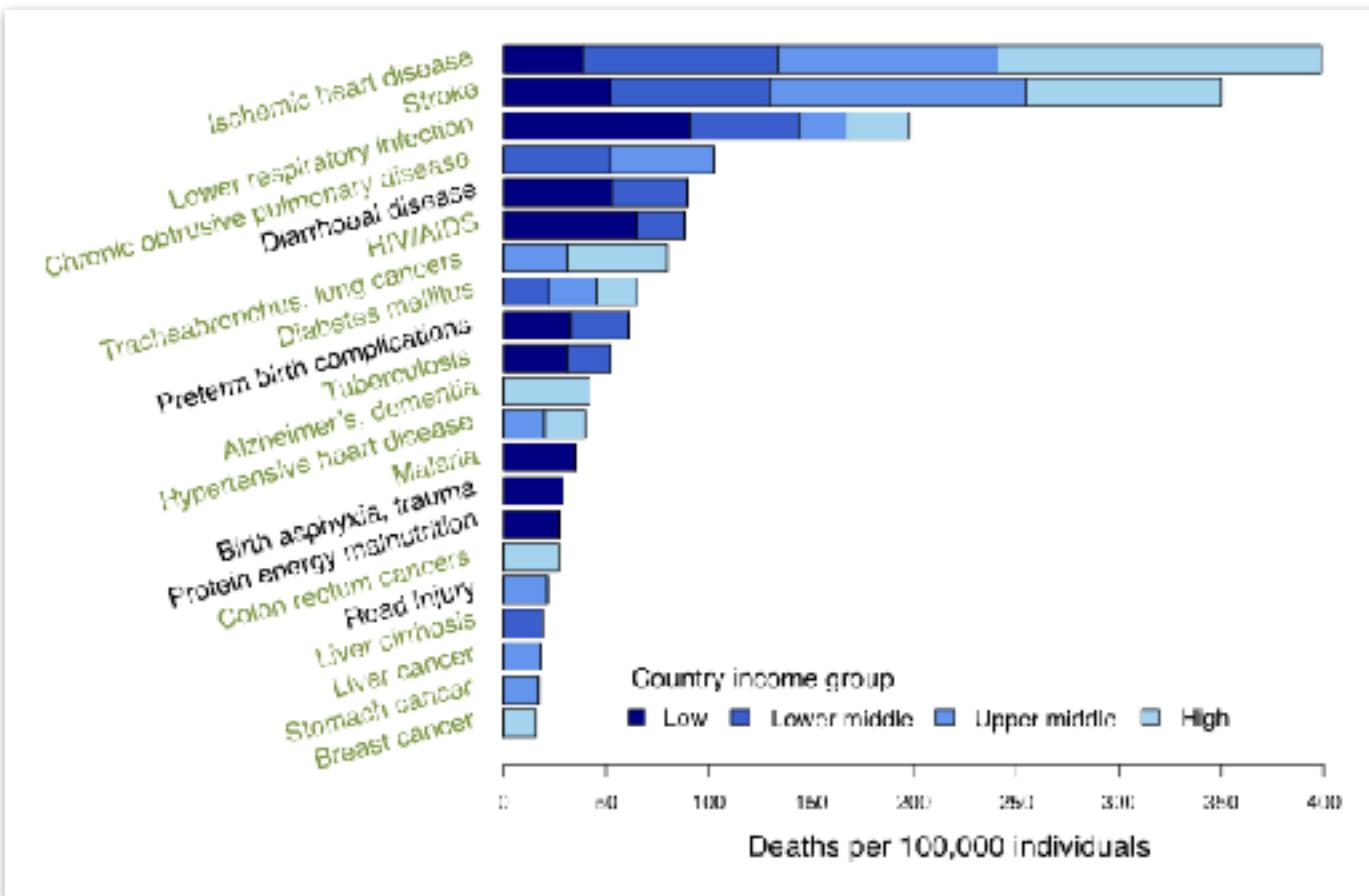


UMC Utrecht
Center for Circulatory Health

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Athero-Express Biobank Studies



Human disease around the globe



The spectrum(s) of disease

Age of onset

Early onset

Late onset

Genetic architecture

One gene

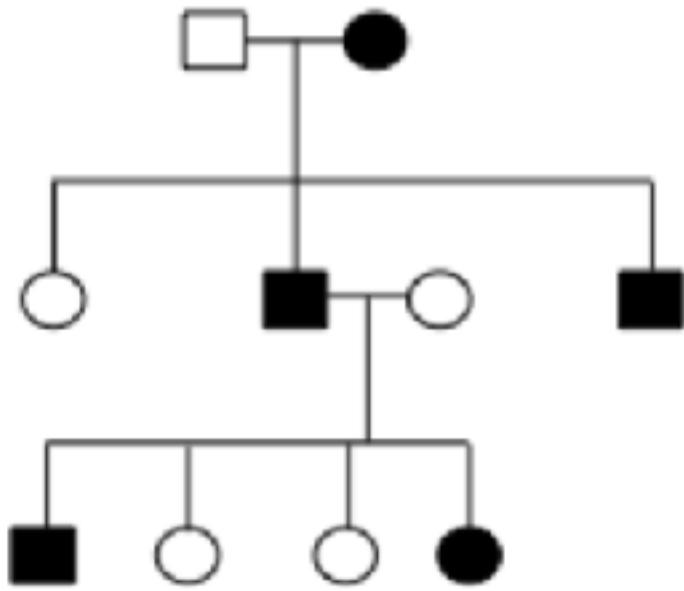
Many genes

Environmental architecture

No role

Major role

Rare (Mendelian) diseases



family-based studies

Genotype



Disease



Environment

Rare (Mendelian) diseases

1983

A polymorphic DNA marker genetically linked to Huntington's disease

James F. Gusella^{*}, Nancy S. Wexler^{†‡}, P. Michael Conneally^{*}, Susan L. Naylor[§], Mary Anne Anderson^{*}, Rudolph E. Tanzi^{*}, Paul C. Watkins[¶], Kathleen Margaret R. Wallace^{*}, Alan Y. Sakaguchi^{||}, Anne B. Young^{||}, Ira S. Ernesto Bonilla^{||} & Joseph B. Martin^{*}

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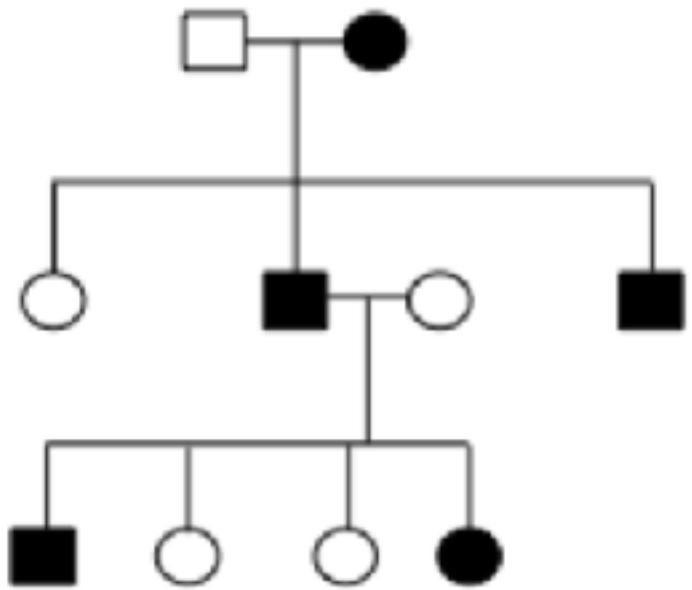
|| Venezuela Collaborative Huntington's Disease Project[¶]

Family studies show that the Huntington's disease gene is linked to a polymorphic DNA marker on chromosome 4. The chromosomal localization of the Huntington's disease gene is the first step in using DNA technology to identify the primary genetic defect in this disorder.



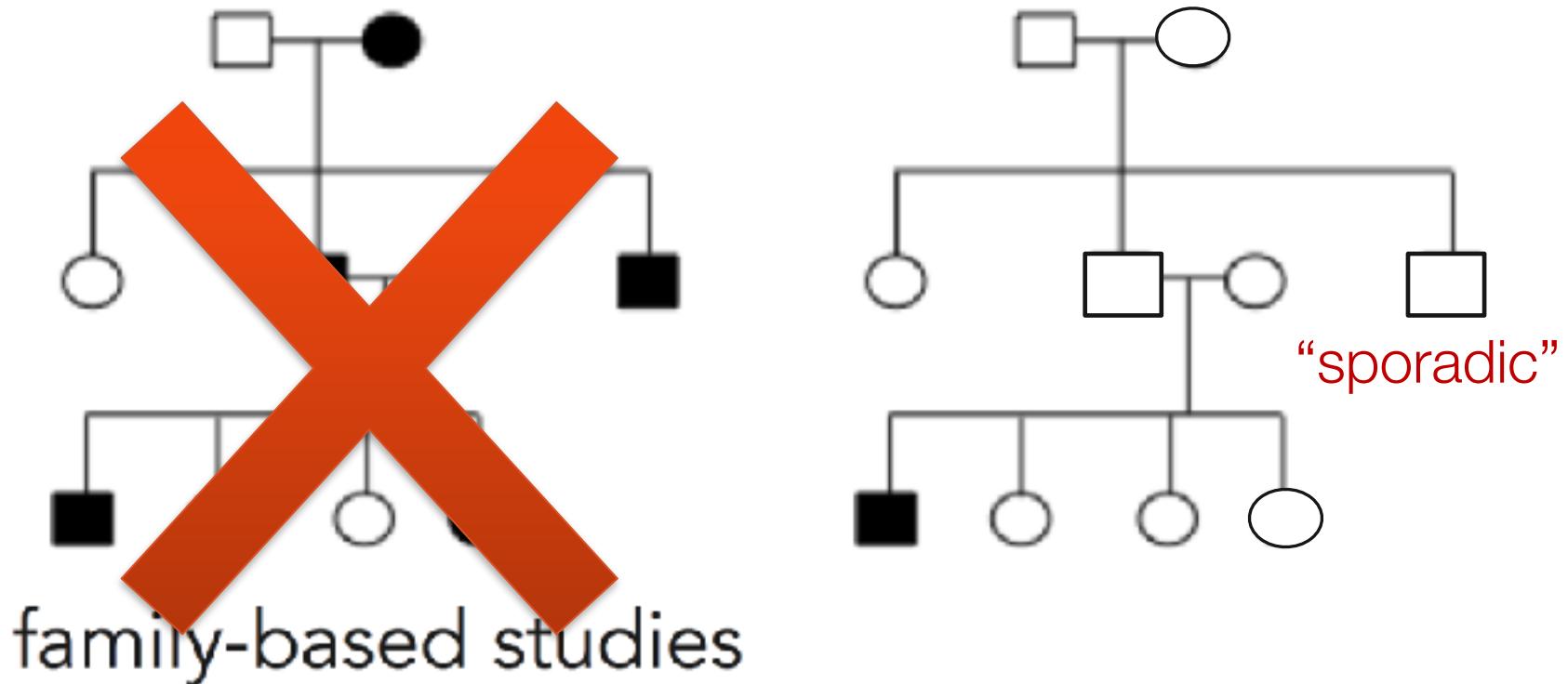
1989

Common (complex) diseases



family-based studies

Common (complex) diseases



family-based studies

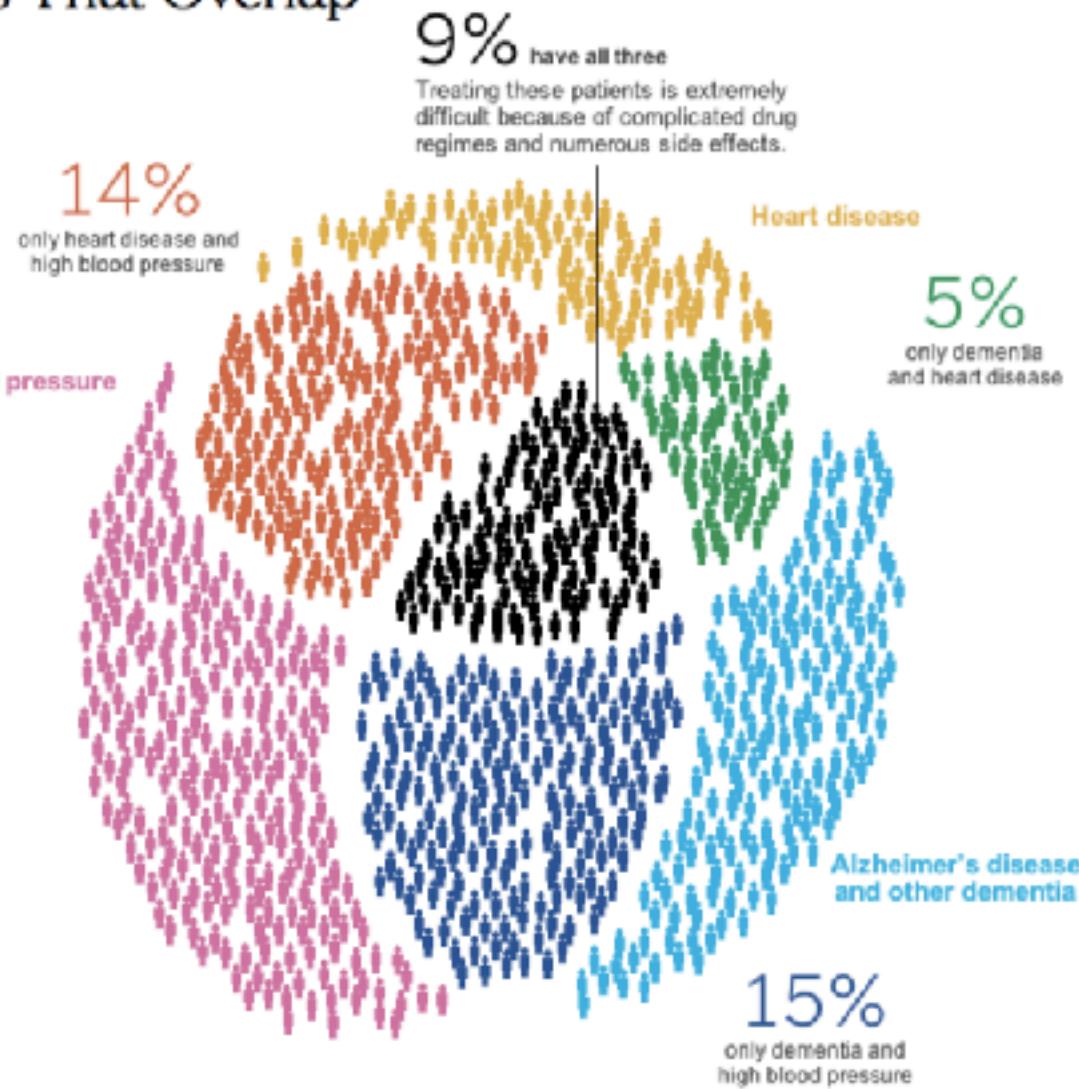
The challenges of common disease

- Heterogeneity
- Late (or broad age range for) onset
- Interaction of genes and environment (multifactorial)
- Overlap with other diseases

For the Elderly, Diseases That Overlap

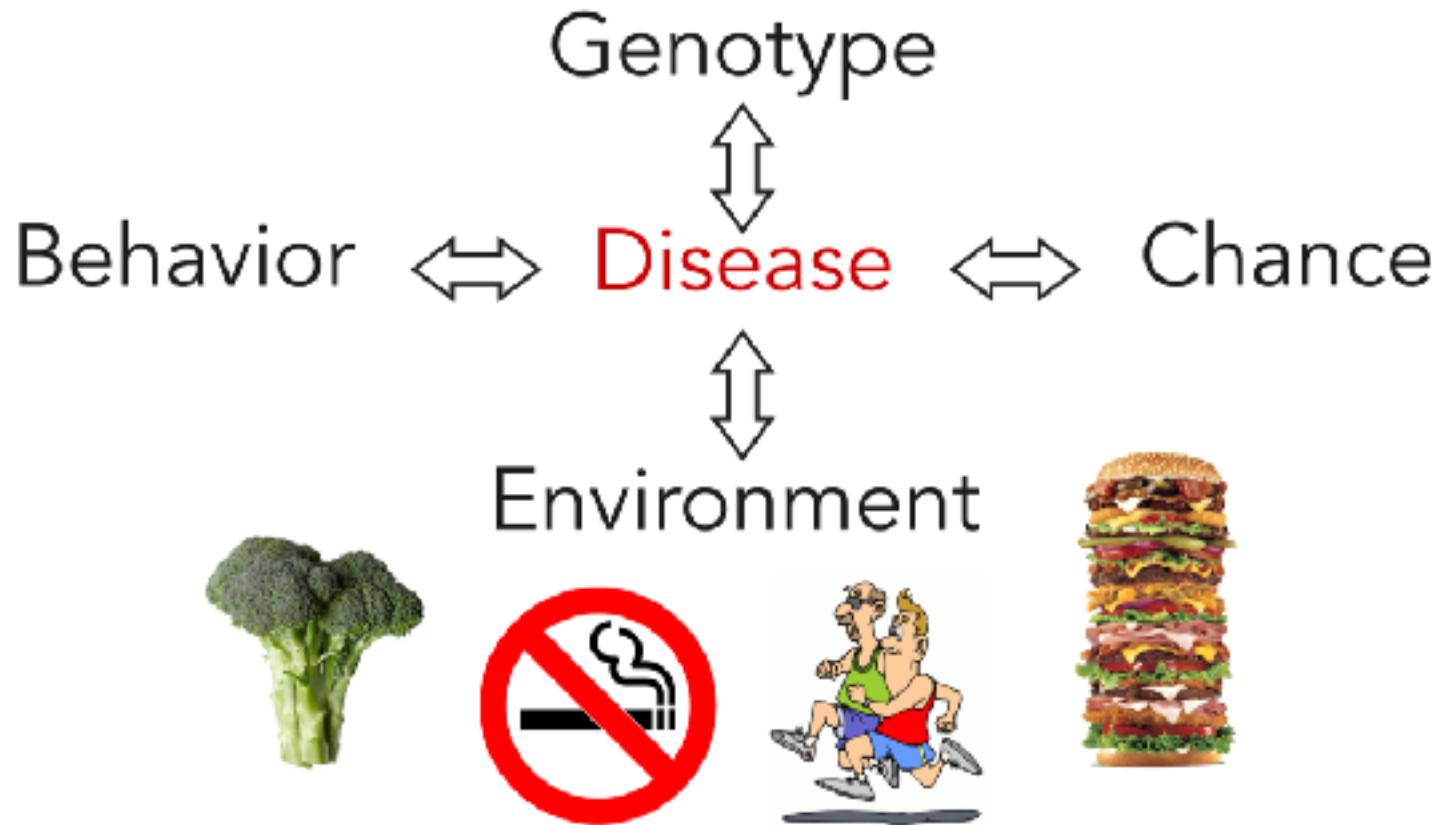
1 2 3 4 5 6 7 NEXT *

Researchers are beginning to focus more intently on the overlaps and possible interconnections, and some scientists argue that it may not be possible to treat dementia without treating vascular problems.

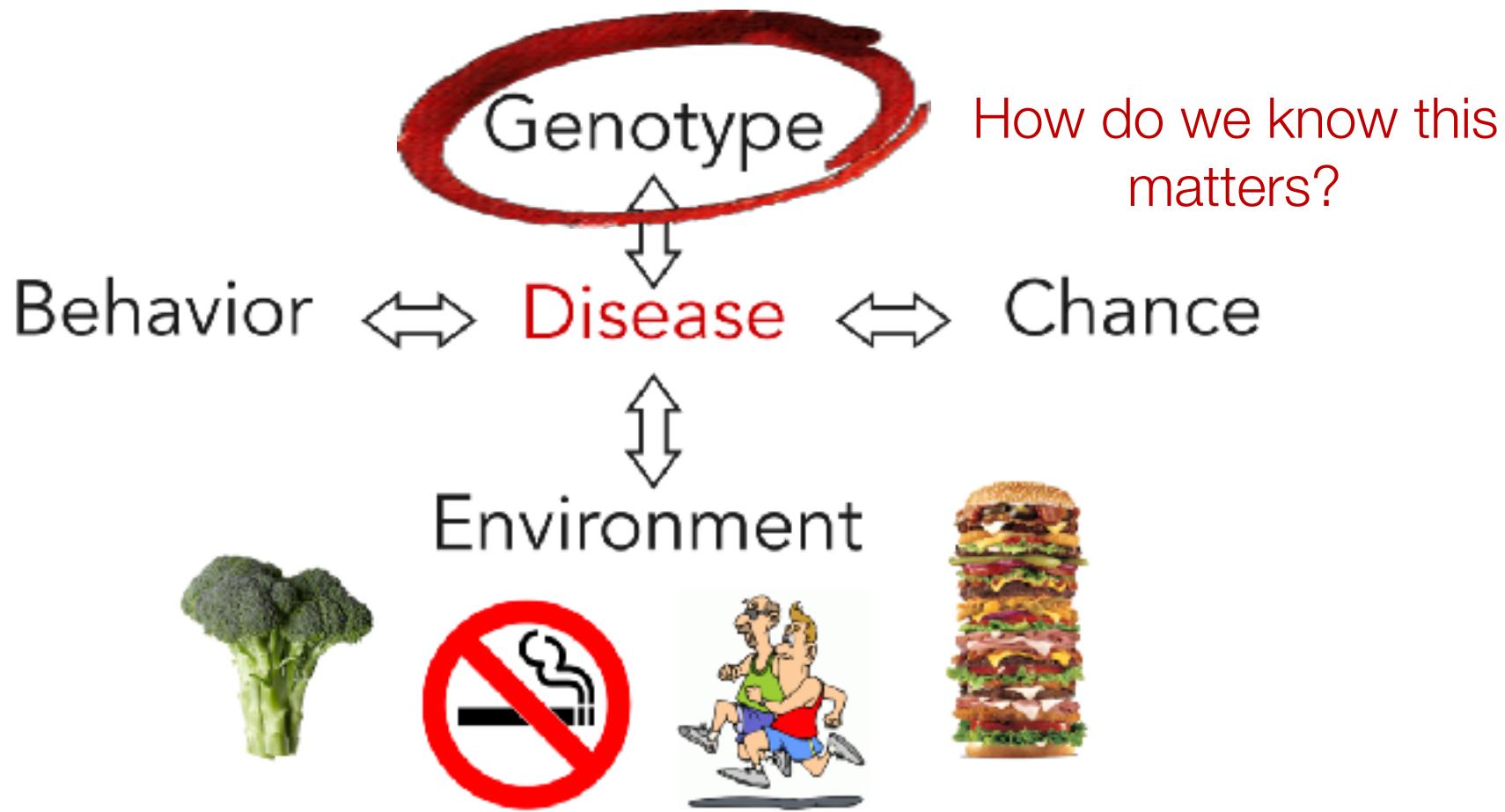


The New York Times, 15 April 2013

Multifactorial disease



Multifactorial disease



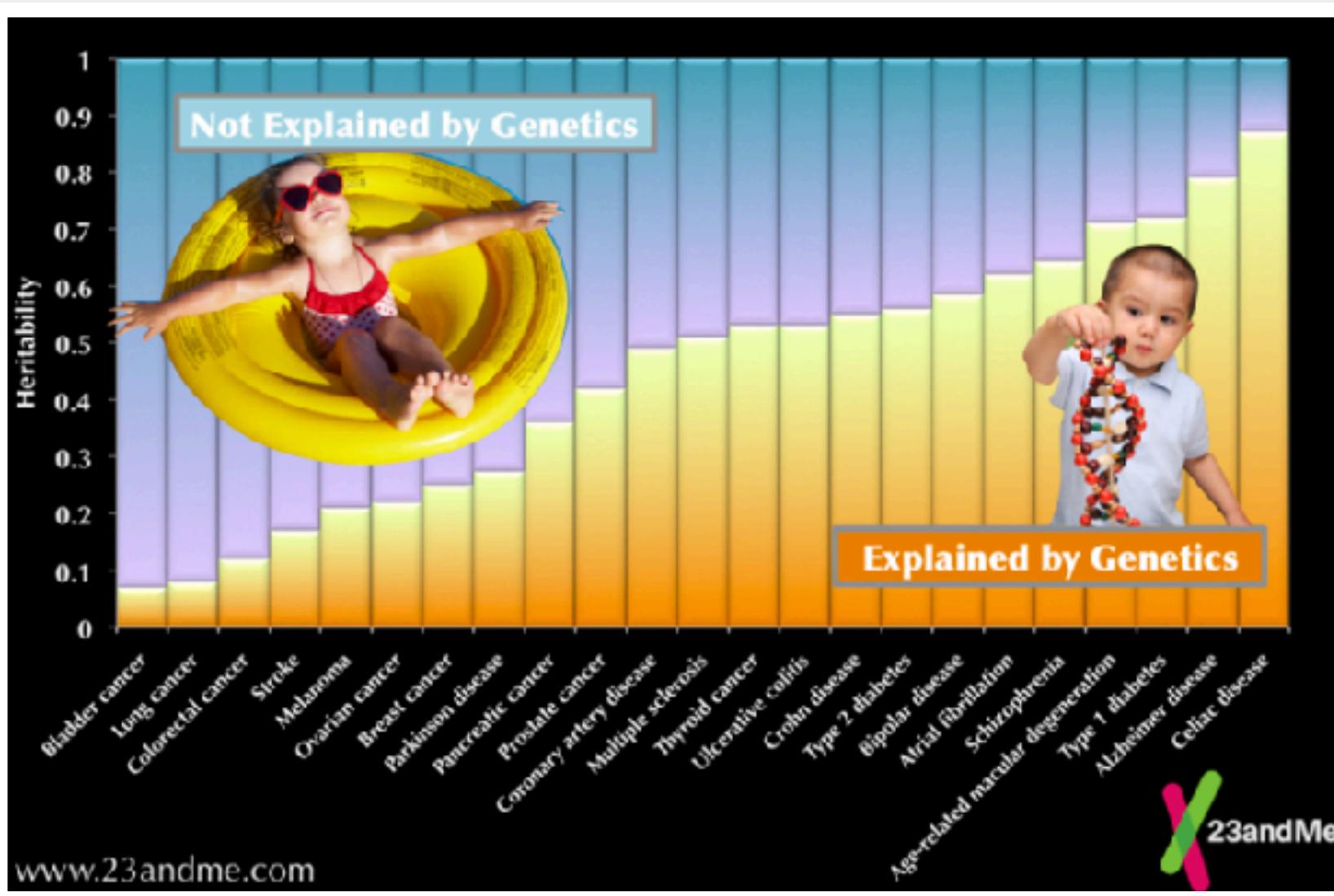


Heritability

Given I am a patient, what is risk of disease for...

	Type 1	Type 2
Your neighbor (unrelated)?	0.4%	5-10%
Your sibling?	6%	30%
Your identical twin?	30-50%	>80%

The range of heritability estimates



Family history

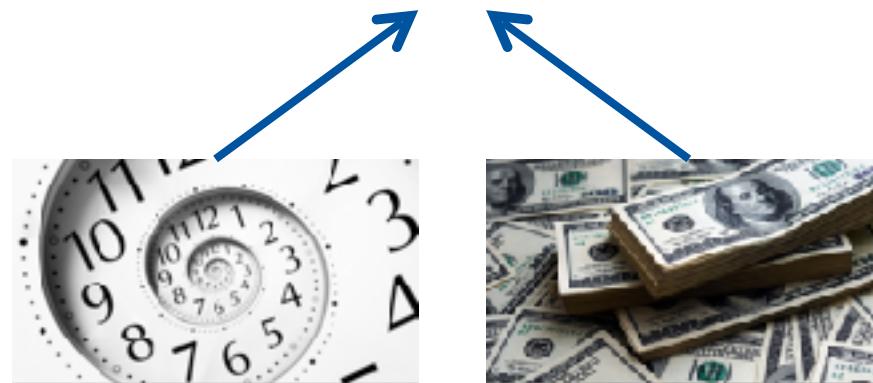
- Framingham Heart Study | www.framinghamheartstudy.org
 - A positive history of cardiovascular disease and associated risk factors tend to aggregate in families
 - Familial aggregation heritability of CVD estimated $\geq 90\%$ (before 46 years)
 - Family history is an independent risk factor (FHS)
 - Positive family history associated with pre-clinical atherosclerosis as measured by carotid IMT, $h^2 \approx 0.35$
- High concordance rate among monozygotic twins, compared to dizygotic twins
- Heritability of atherosclerosis (carotid IMT) $h^2 \approx 0.21-0.64$ and is increased by age and cardiovascular risk factors

There is clearly a heritability factor for atherosclerotic and consequent cardiovascular disease

Why do some individuals have a higher risk for a disease than others?

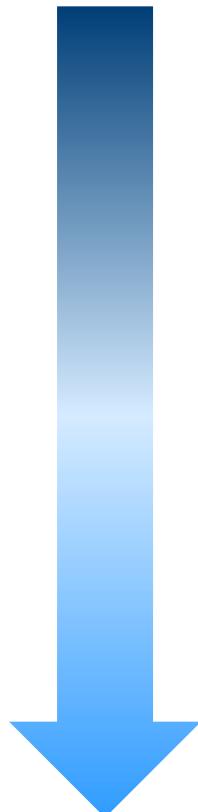
How can we alleviate disease burden in the human population?

Drug development



What's the goal of genetics?

- Understanding true causal disease pathways
 - Identify risk factors
 - Inform novel research directions
 - Enable rational and efficient drug development
- Precision medicine
 - Evaluate individual disease risk
 - Early disease identification or prevention
 - Understand patient's therapeutic response



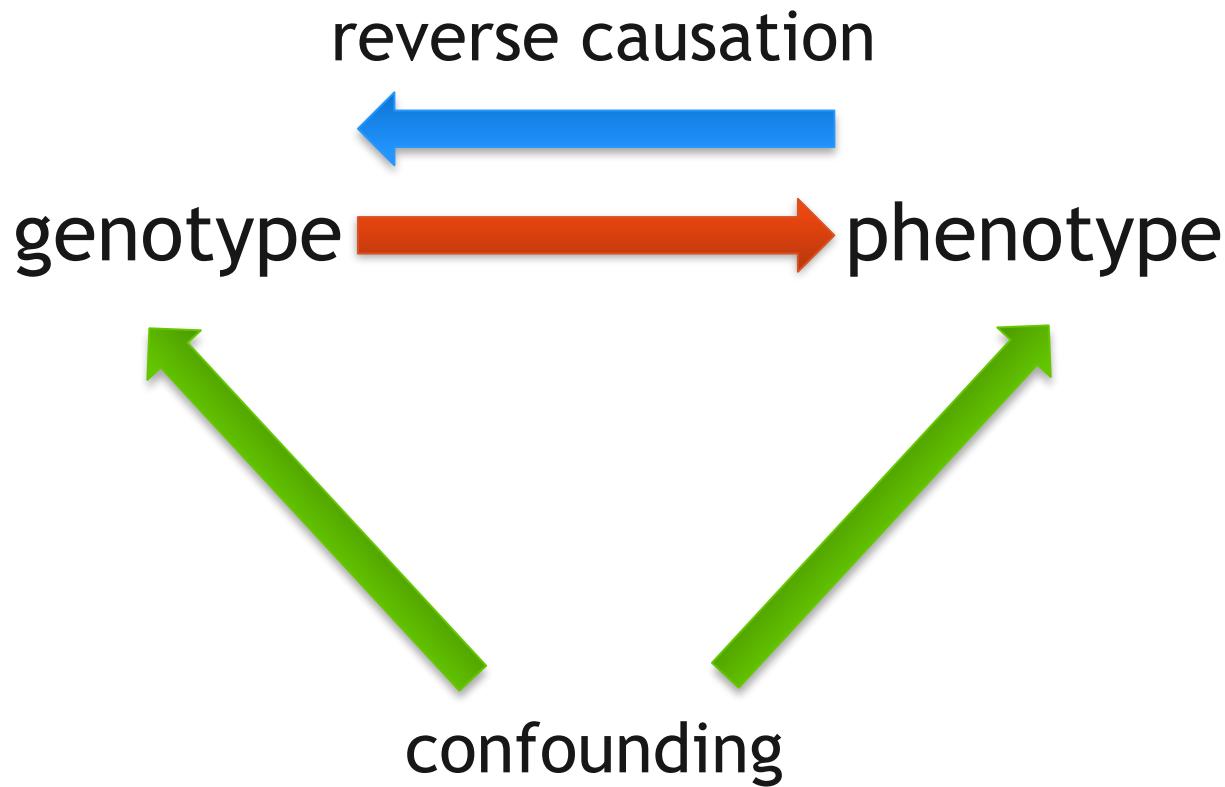
Why genetics at all?

- Genotypes are randomly assigned at meiosis
 - Nature's randomized clinical trial
- Genotypes are fixed and unaltered by the disease
 - Exception: somatic mutations in cancer
- We have become increasingly good at measuring genotypes
 - Lots and lots of data

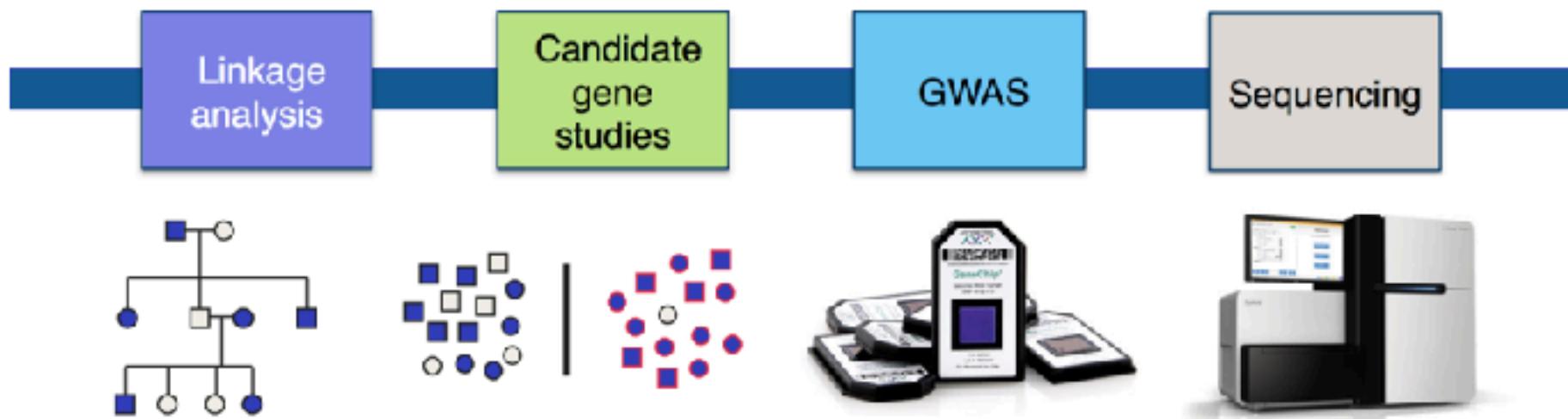
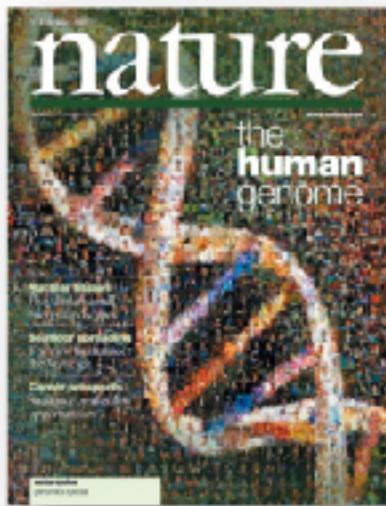
The limitations of genetics

genotype  phenotype

The limitations of genetics

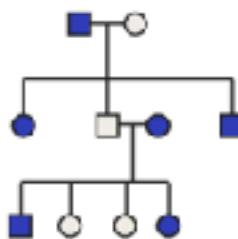


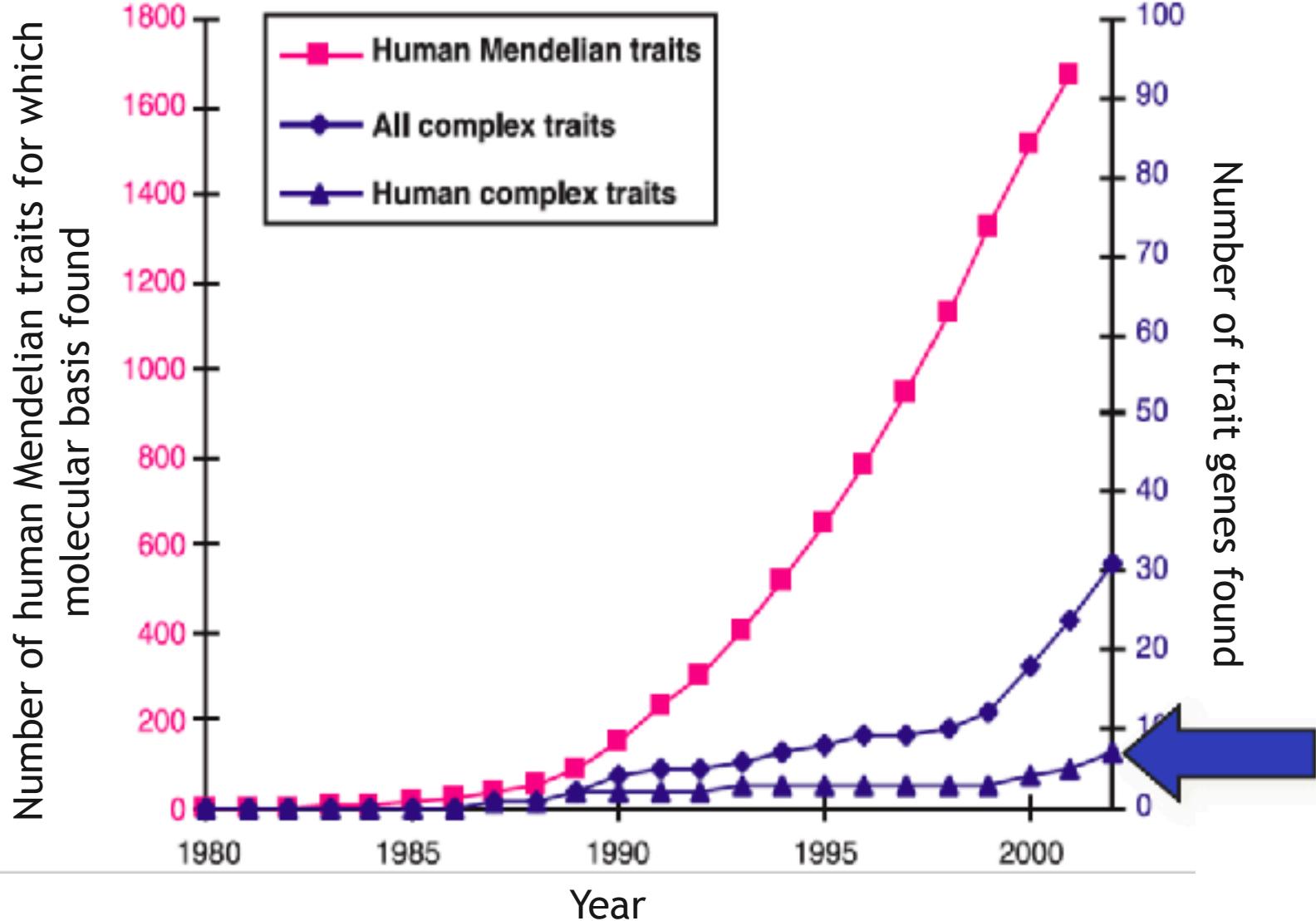
Where we've been and where we are

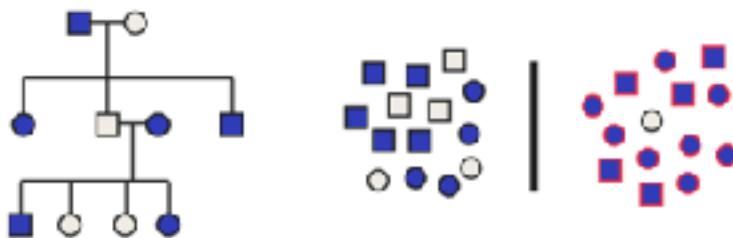
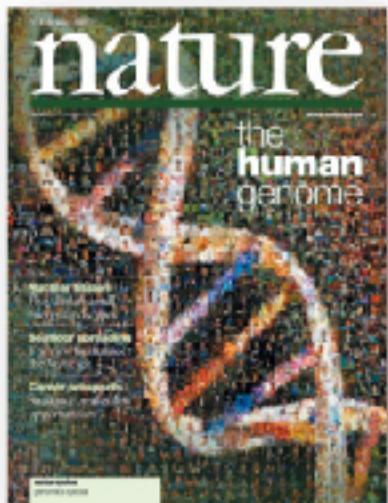




Linkage analysis







The candidate gene approach

- Pick a gene that might have a role in your disease
arbitrary
- Genotype individuals at a few sites around that gene
 - Typically 1,000 - 2,000 samples
no power
- Test genetic sites for association

A poor history of candidate gene studies

March/April 2002 • Vol. 4 • No. 2

review

A comprehensive review of genetic association studies

Joel N. Hirschhorn, MD, PhD^{1,2}, Kirk Lohmueller¹, Edward Byrne¹, and Kurt Hirschhorn, MD³

Most common diseases are complex genetic traits, with multiple genetic and environmental components contributing to susceptibility. It has been proposed that common genetic variants, including single nucleotide polymorphisms (SNPs), influence susceptibility to common disease. This proposal has begun to be tested in numerous studies of association between genetic variation at these common DNA polymorphisms and variation in disease susceptibility. We have performed an extensive review of such association studies. We find that over 600 positive associations between common gene variants and disease have been reported; these associations, if correct, would have tremendous importance for the prevention, prediction, and treatment of most common diseases. However, most reported associations are not robust: of the 166 putative associations which have been studied three or more times, only 6 have been consistently replicated. Interestingly, of the remaining 160 associations, well over half were observed again one or more times. We discuss the possible reasons for this irreproducibility and suggest guidelines for performing and interpreting genetic association studies. In particular, we emphasize the need for caution in drawing conclusions from a single report of an association between a genetic variant and disease susceptibility. *Genet Med* 2002;4(2):45–61.

Key Words: human genetics, association studies, common disease, polymorphisms

Why Most Published Research Findings Are False

John P. A. Ioannidis

PloS Medicine, 2005

The candidate gene problem:

- Lack of statistical rigor
- Lack of large samples
- Lack of data quality control
- Lack of replication data

Need systematic, unbiased approach

Problems with the candidate gene approach

- Small sample sizes
- Weak effects
- No community-wide standards for QC, association claims
- Population stratification

Important side note: this still happens

OPEN  ACCESS Freely available online

PLOS GENETICS

AVPR1a and SLC6A4 Gene Polymorphisms Are Associated with Creative Dance Performance

Psychiatr Q (2014) 85:257–265
DOI 10.1007/s11126-013-9387-x

ORIGINAL PAPER

The 2-Repeat Allele of the MAOA Gene Confers an Increased Risk for Shooting and Stabbing Behaviors



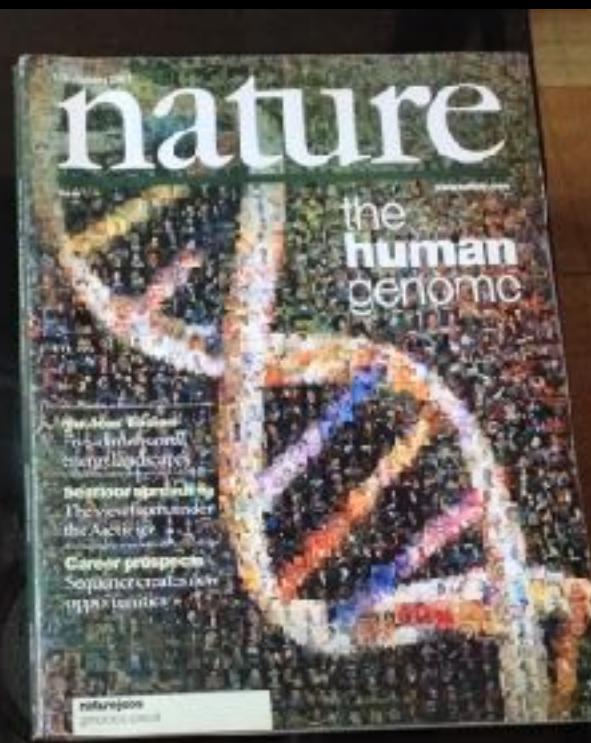
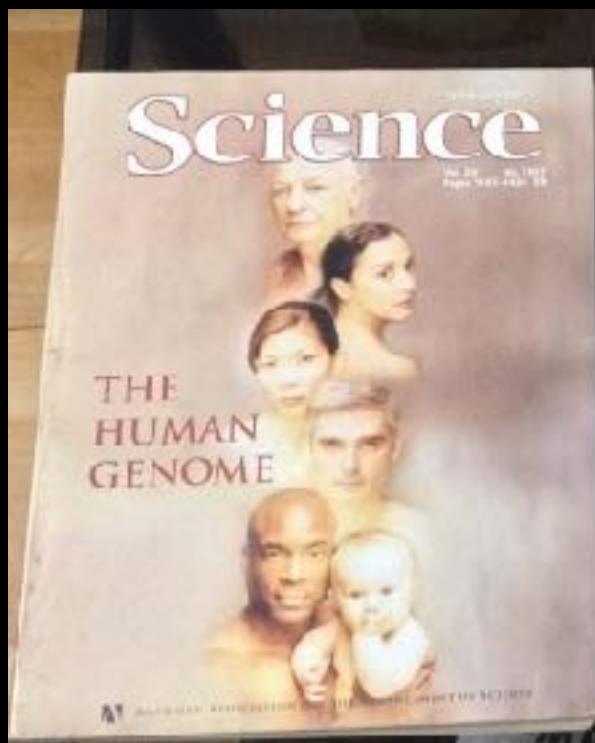
OPEN The association between romantic relationship status and 5-HT1A gene in young adults

SUBJECT AREAS:
HUMAN BEHAVIOR
BEHAVIORAL GENETICS

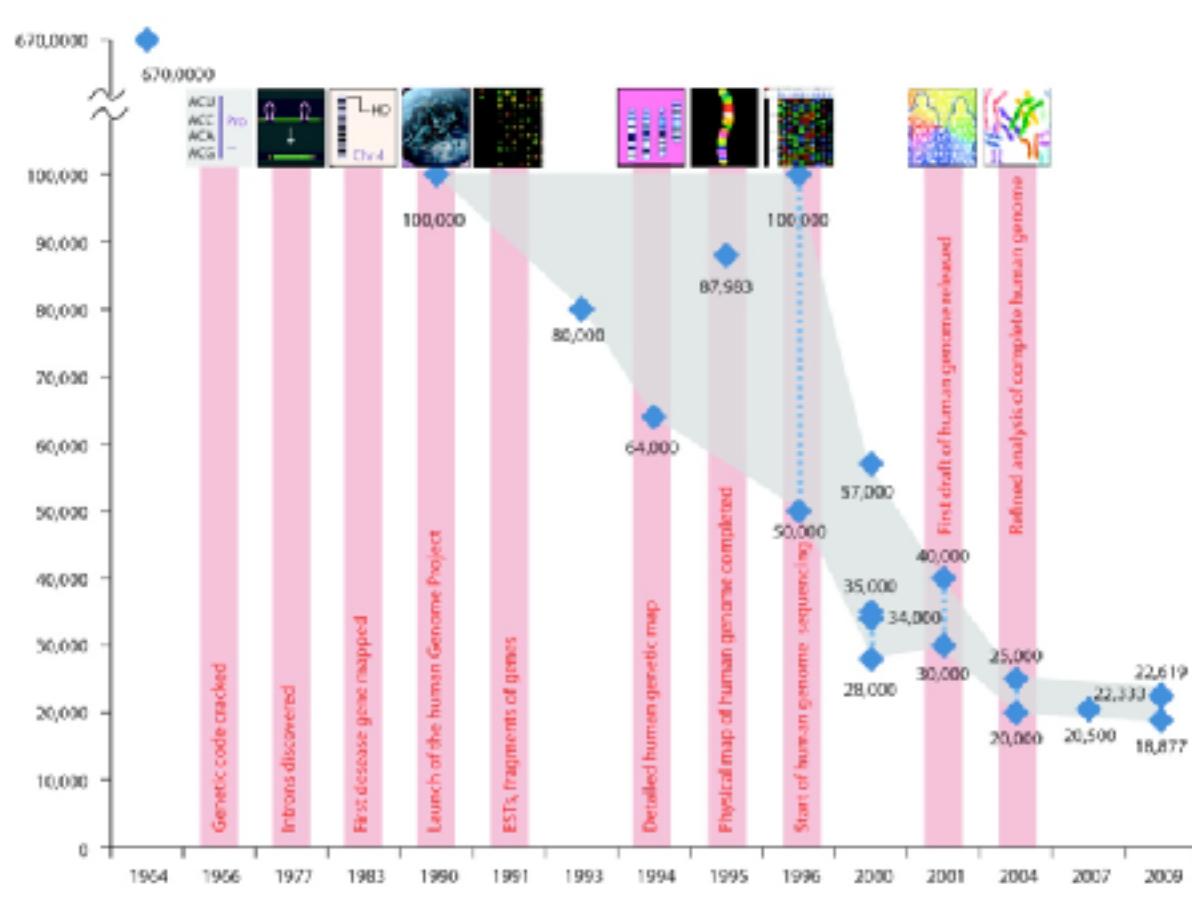




The Human Genome Project “paid forward” and paved the way for modern day genomics

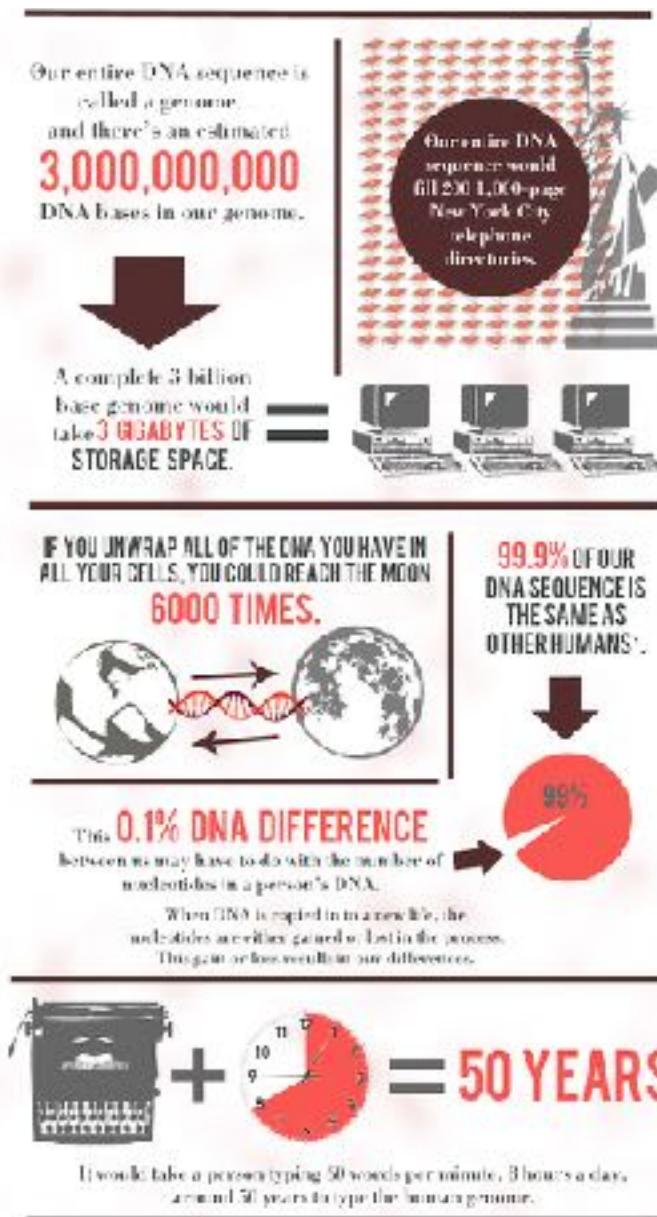


(Finally) a complete map



Human Genome: *some statistics*

- 3.2 billion base pairs in the haploid genome
- ≈ 18,000-25,000 genes
 - ≈23,000 coding for proteins
 - Only 1.5% of the total genome
- Rest of the genome:
 - Non-coding RNA (rRNA, tRNA)
 - Regulatory sequences, e.g. promoter, enhancer regions
 - Repetitive elements and other variations
 - Transposable elements
- (So there's no such thing as “junk DNA”...)



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ACATATAATG
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CACATTTCG

Most of genetic variation is due to *single nucleotide polymorphisms (SNPs)* --single base changes that are common in the general population

Human genome: *individual variations*

- Human genome is ~99 % similar between individuals
- 0.5-1% different

articles

A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms

The International SNP Map Working Group*

* A full list of authors appears at the end of this paper.

We describe a map of 1.42 million single nucleotide polymorphisms (SNPs) distributed throughout the human genome, providing an average density on available sequence of one SNP every 1.9 kilobases. These SNPs were primarily discovered by two projects: The SNP Consortium and the analysis of clone overlaps by the International Human Genome Sequencing Consortium. The map integrates all publicly available SNPs with described genes and other genomic features. We estimate that 60,000 SNPs fall within exon (coding and untranslated regions), and 85% of exons are within 5 kb of the nearest SNP. Nucleotide diversity varies greatly across the genome, in a manner broadly consistent with a standard population genetic model of human history. This high-density SNP map provides a public resource for defining haplotype variation across the genome, and should help to identify biomedically important genes for diagnosis and therapy.

The International HapMap Project

Phase I

1.1 million SNPs

270 individuals from 4 populations



Phase II

3.1 million SNPs

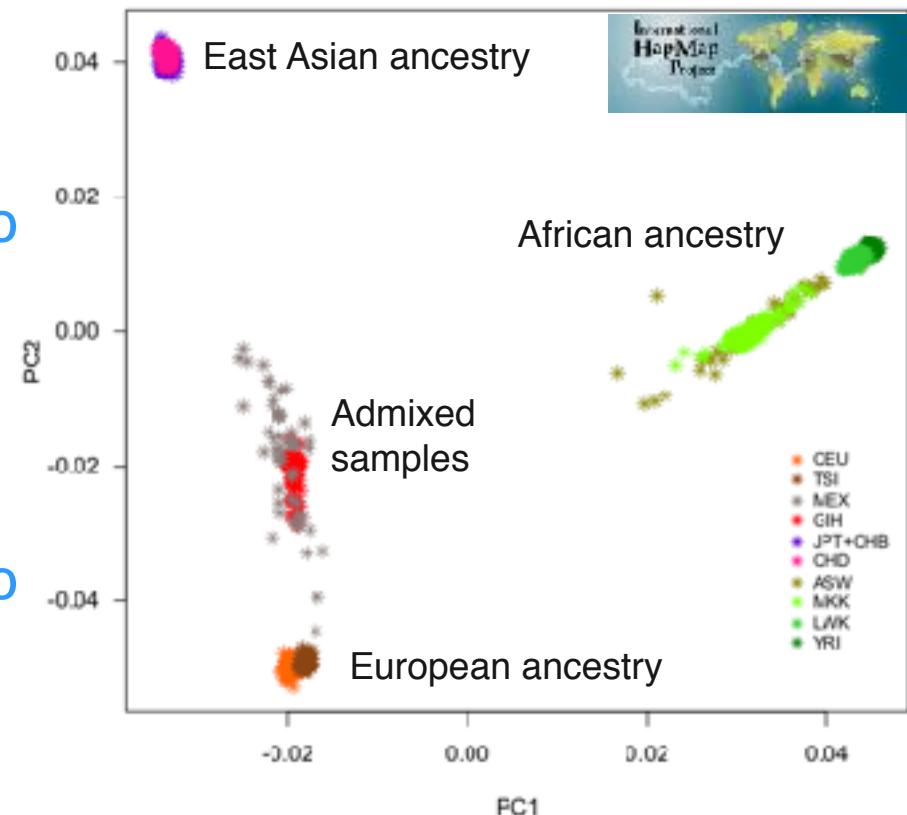
270 individuals from 4 populations



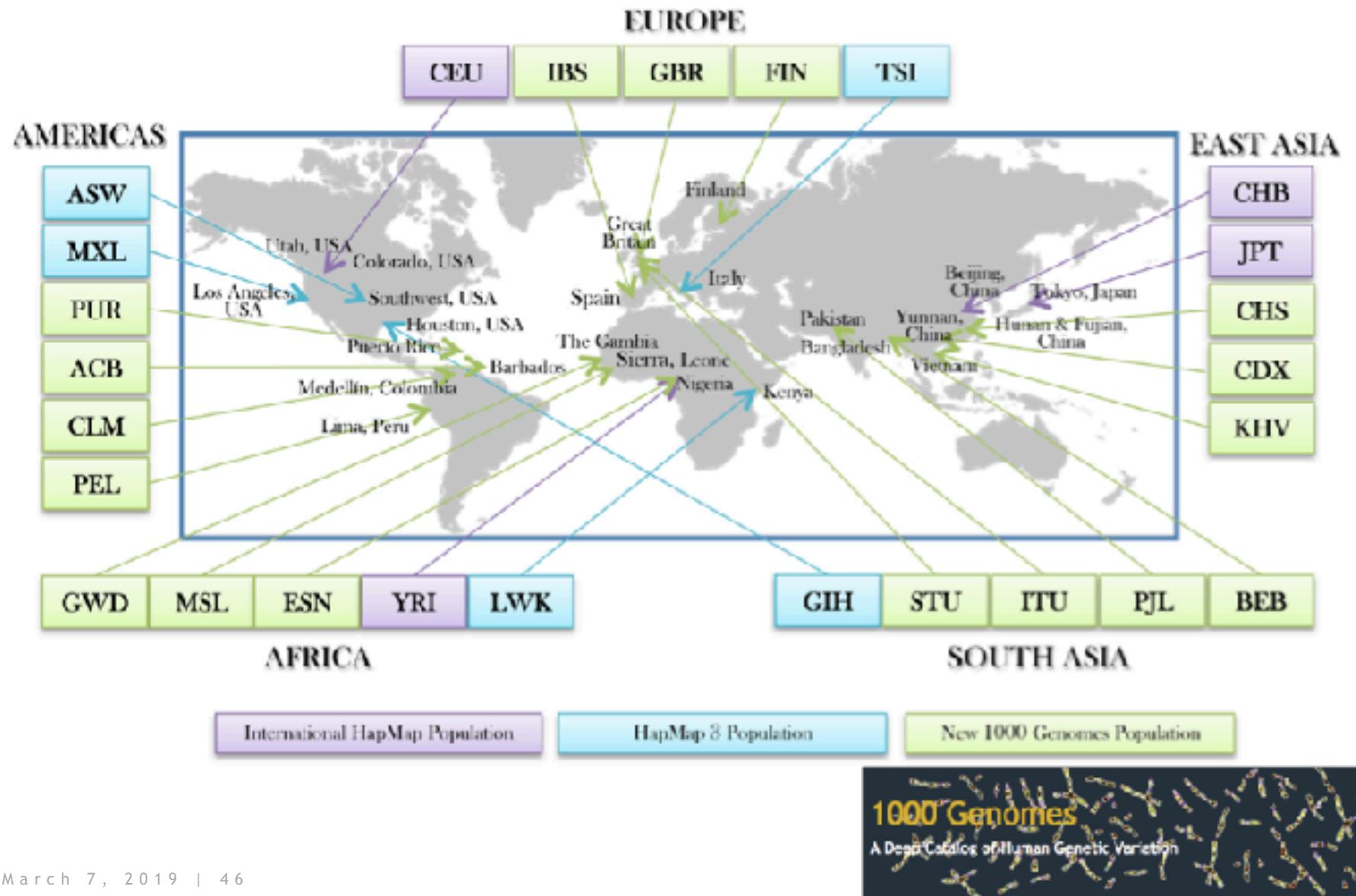
Phase III

1.6 million SNPs

1,184 individuals from 11 populations



The 1000 Genomes Project

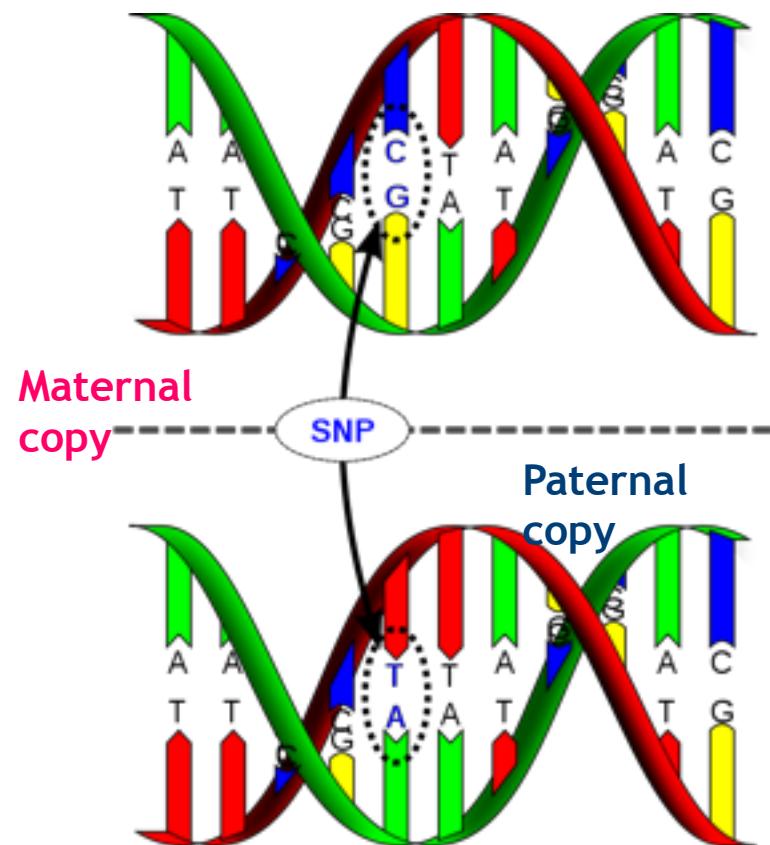


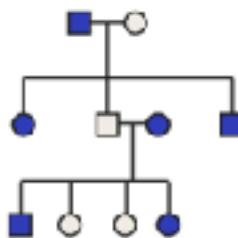
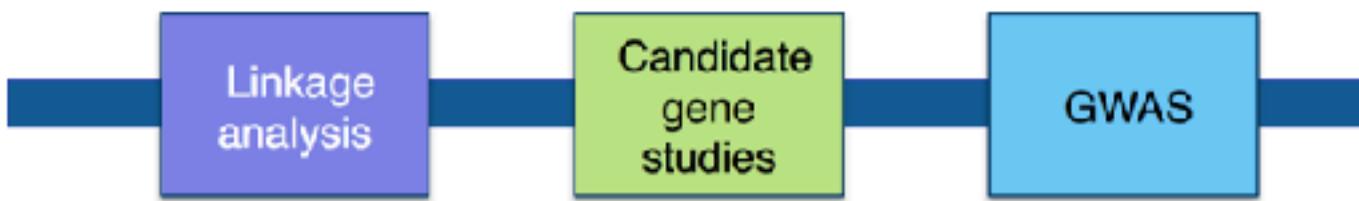
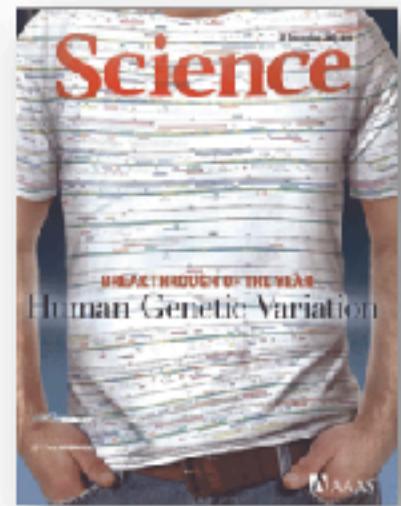
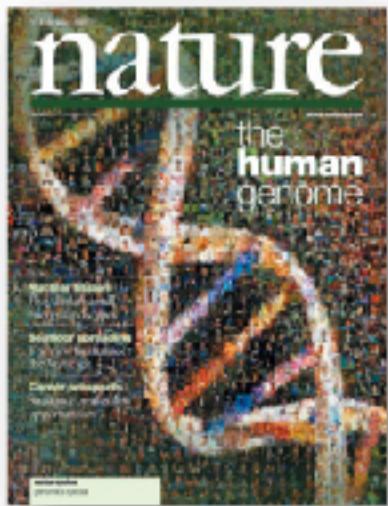
Single-Nucleotide Polymorphism

- “one base pair variation”
 - > 1% general population
(common)
 - ≈10 million SNPs (≈0.25% genome)
 - Makes you and me unique
 - Most common type of genetic variation



www.hapmap.org





Common variant, common disease hypothesis

- Most common diseases happen later in life
- If common variants are not selected against, they may associate to late-onset (after reproduction) disease
- Common variants are easier to find and characterize

The beginnings of GWAS

HapMap Phase I

HapMap Phase II

SNP arrays

WTCCC GWAS

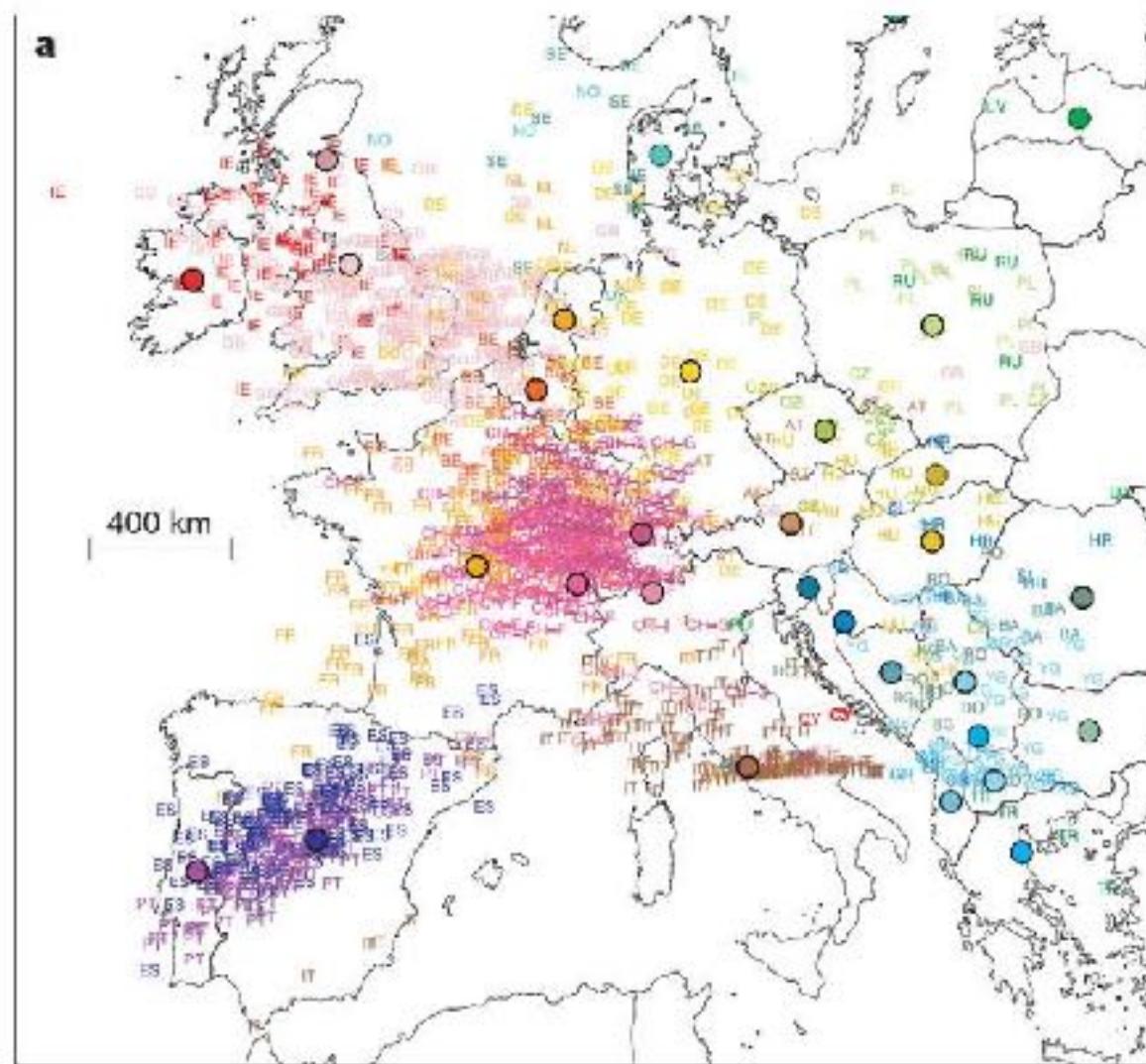
HapMap Phase III



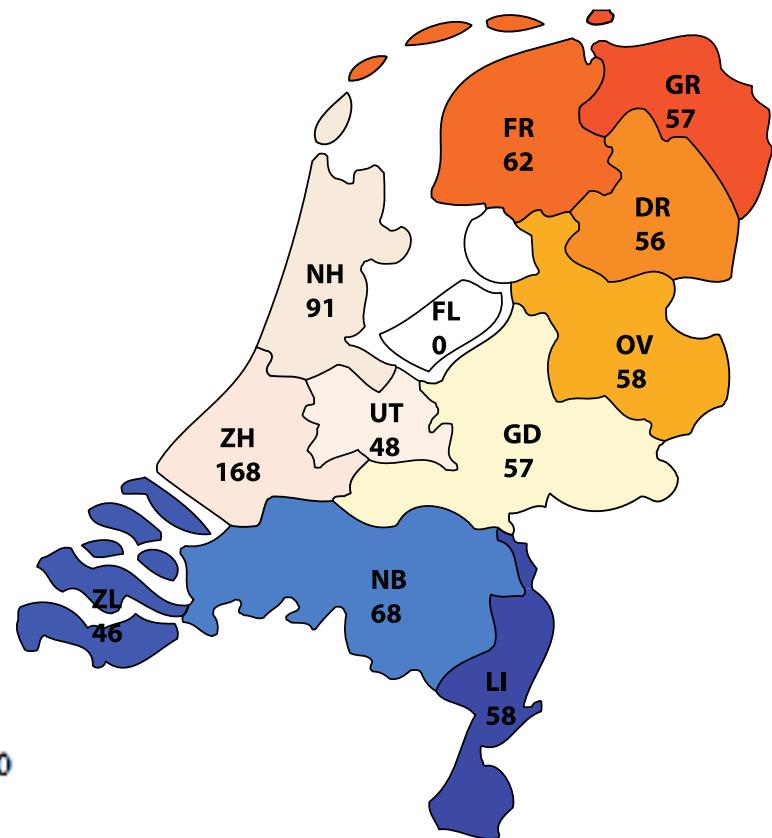
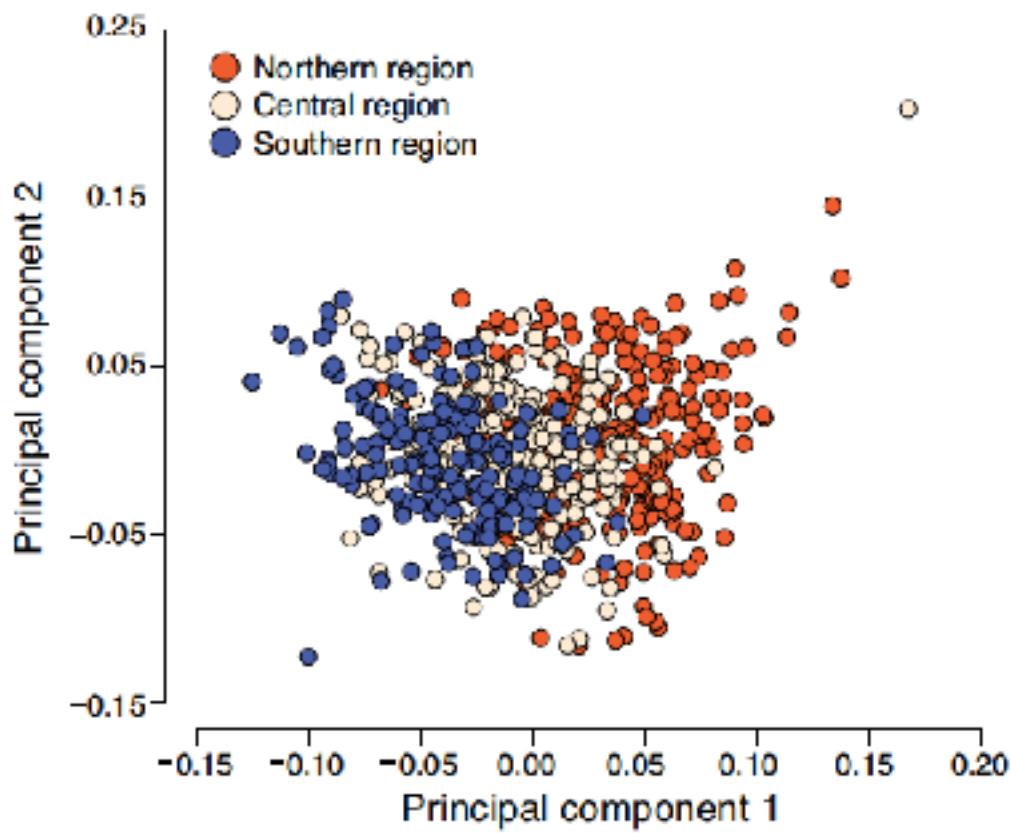
2003

2010

Differentiation of populations



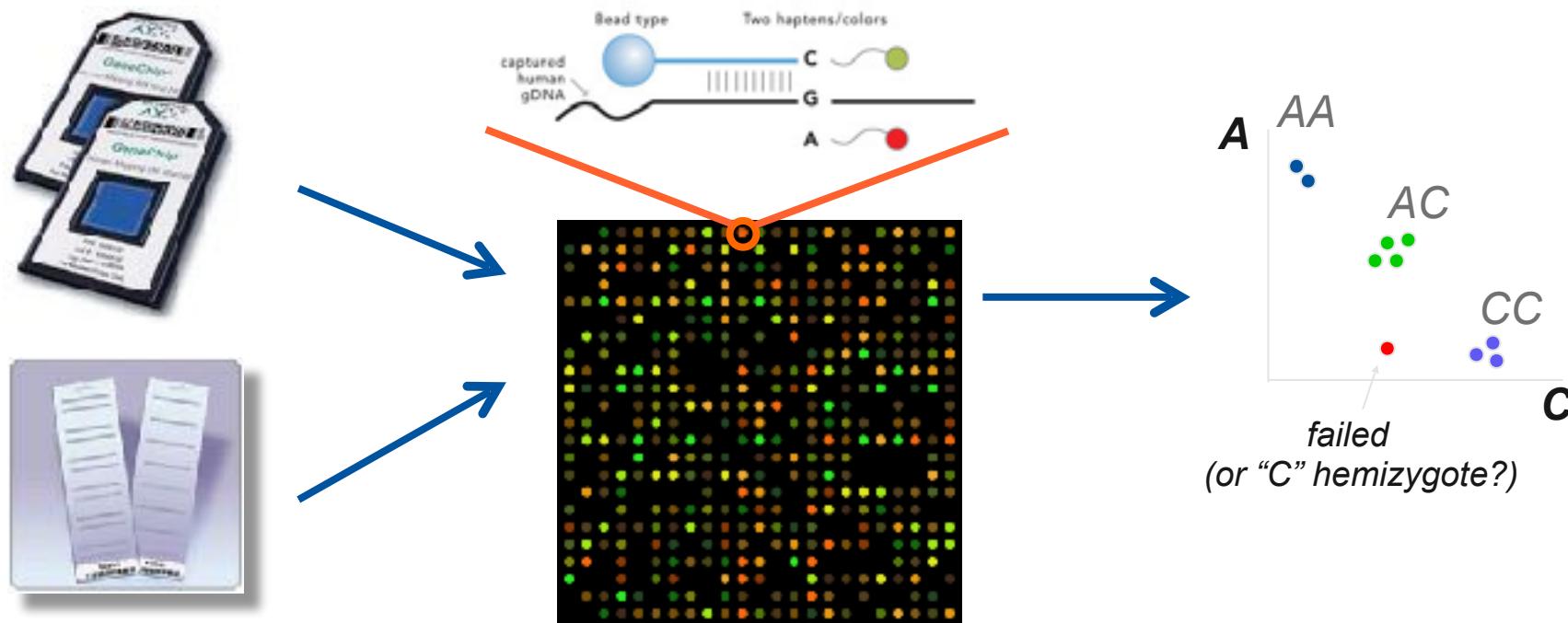
Within the Netherlands, North-South substructure



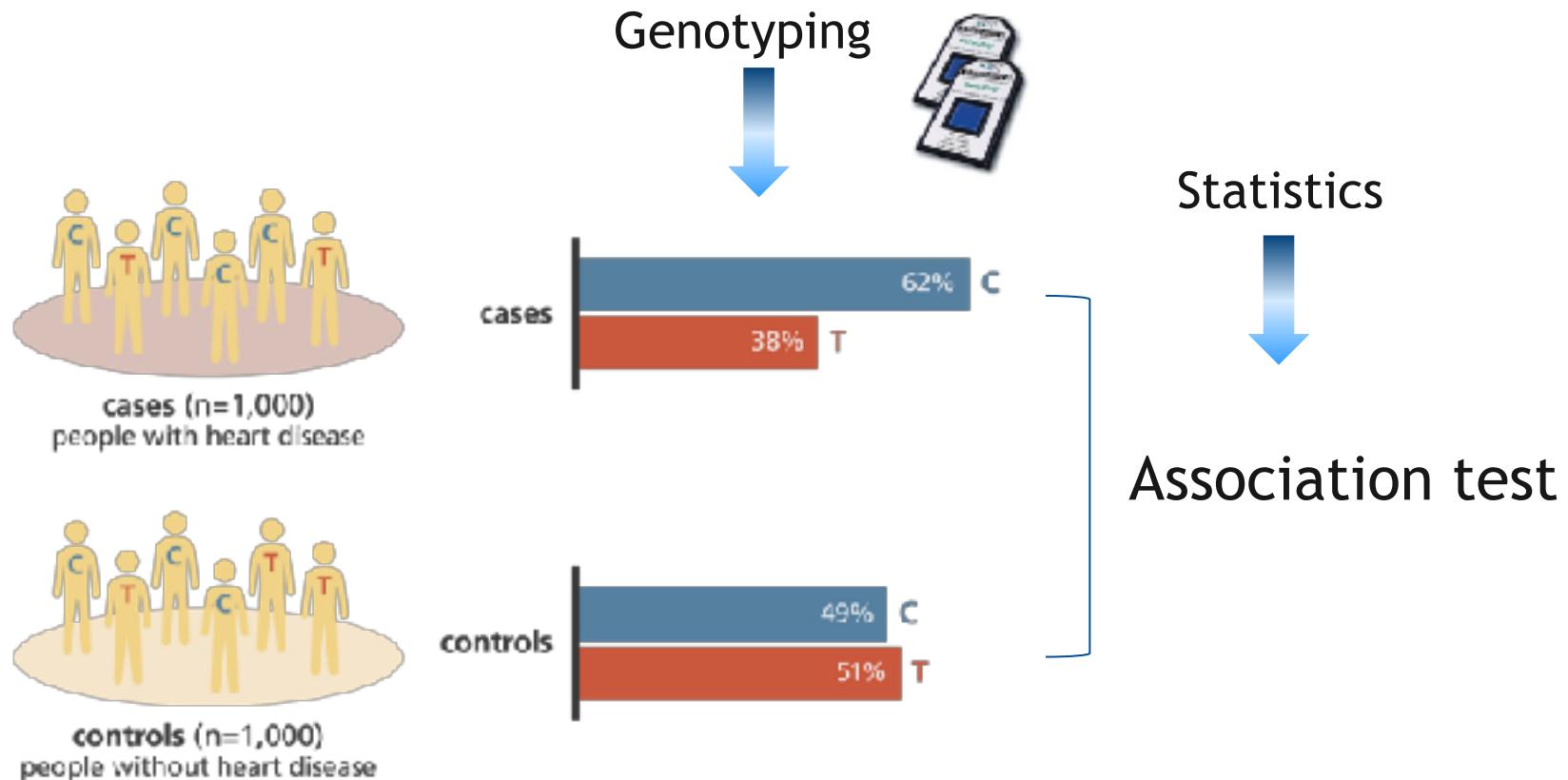
Genome of the Netherlands (250 families whole-genome sequenced)

Genotyping platforms

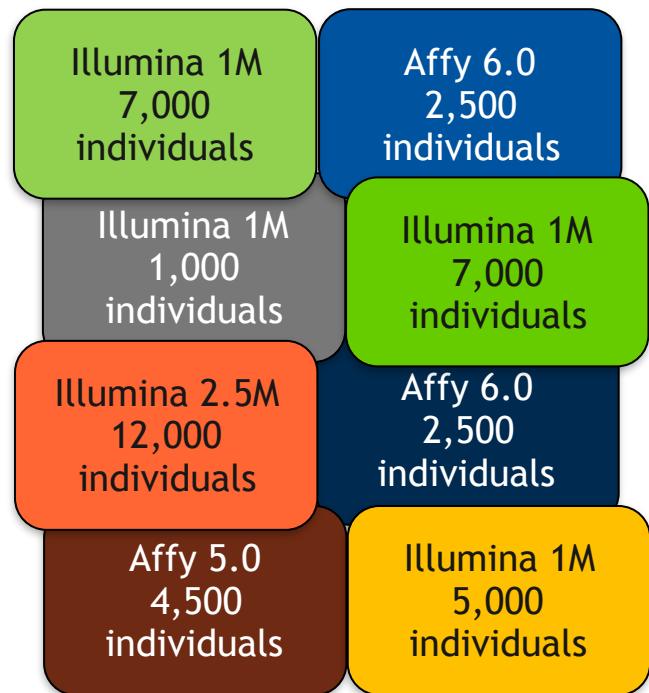
- Genome-wide SNP microarrays allow measurement of genotypes of 100,000's of SNPs in a single experiment
- Variety of microarrays (different SNP density, cost, etc) by Illumina and Affymetrix



GWAS (the big picture)

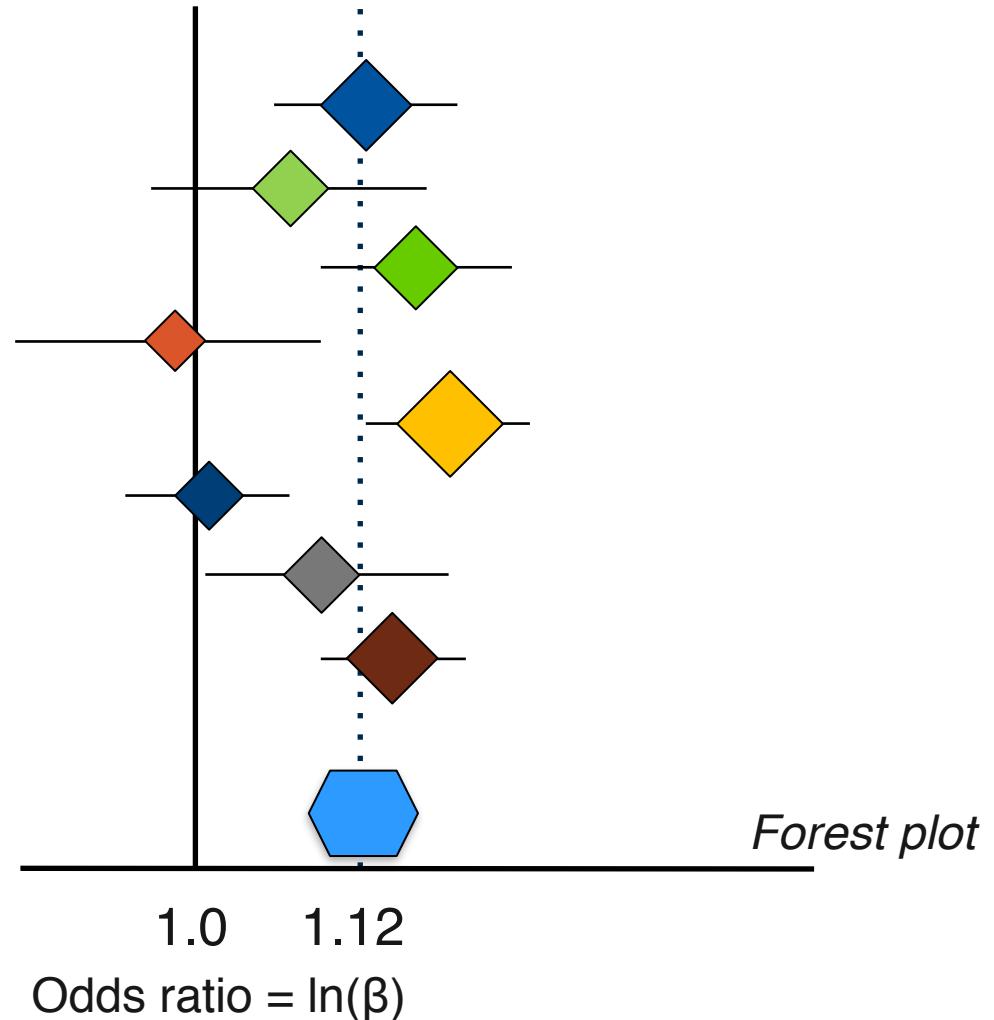


Combining GWAS datasets



Imputation
↓
Meta-analysis of GWAS

Results for one SNP



deCODE Genetics, Inc.

- >50% adult population of Iceland (>140,000) in biobank (blood)
- Pedigree information going back to the first settlements (\approx 1000 years ago)
- Extensive medical records & genotypic data
- Over 250 high-impact publications (Nature, Science, ...)
- 50 common diseases
 - Stroke (=CVA) association with *ALOX5AP*
 - MI association with *ALOX5AP*
 - Association of a variant on 9p21.1 with Abdominal aortic aneurysm (AAA), intracranial aneurysm, stroke and MI



The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke

Aruna Helgadottir¹, Andre Masselink¹, Gudmar Thorleifsson¹, Sigrun Grotundottir¹, Hlynur Ingvarsson¹, Unnur Thorsdottir¹, Sigrun J. Sigurdardottir¹, Guðrún Ólafsdóttir¹, Steinn J. Gudjonsson¹, Elinor M. Vidalineau¹, Rögnvaldur E. Margeirsson¹, Rúnar Johannesson¹, Ólafur Ólafsson¹, Marta Horwitz², Jonathan A. Tager-Flusberg², Margaret Thorleifsson¹, Michael E. Fager³, Eric J. Topper³, Argentine Kong⁴, Vilma Ólafsdóttir¹, Ásthildur Hjartarsdóttir¹, Jeffrey C. Dahlöf⁵ & Kari Stefansson¹

We mapped a gene predisposing to myocardial infarction to a locus on chromosome 1q–2. This major single nucleotide polymorphism (SNP) haplotype in this locus spanning the gene *ALOX5AP* encoding a 5-lipoxygenase activating protein (Lipoxin A4) is associated with the baseline gender and age stratified risk for infarct. The haplotype confers an increased two-fold greater risk of earlier stroke. ALOX5AP haplotypes were tested with a similar risk score in individuals from the UK Biobank cohort consisting of individuals with a myocardial infarction probability (MI) as low as predicted in the 5-lipoxygenase pathway, thus downgrading from stroke, and this difference was largely attributed to cells from males who carry the atherosclerosis risk variants that consist of *ALOX5AP* as involved in the pathogenesis of both myocardial infarction and stroke by increasing leukocyte production and infiltration in the arterial wall.

Helgadottir, A., et al. *Nature Genetics*; volume 36, 233; 2004

A Common Variant on Chromosome 9p21 Affects the Risk of Myocardial Infarction

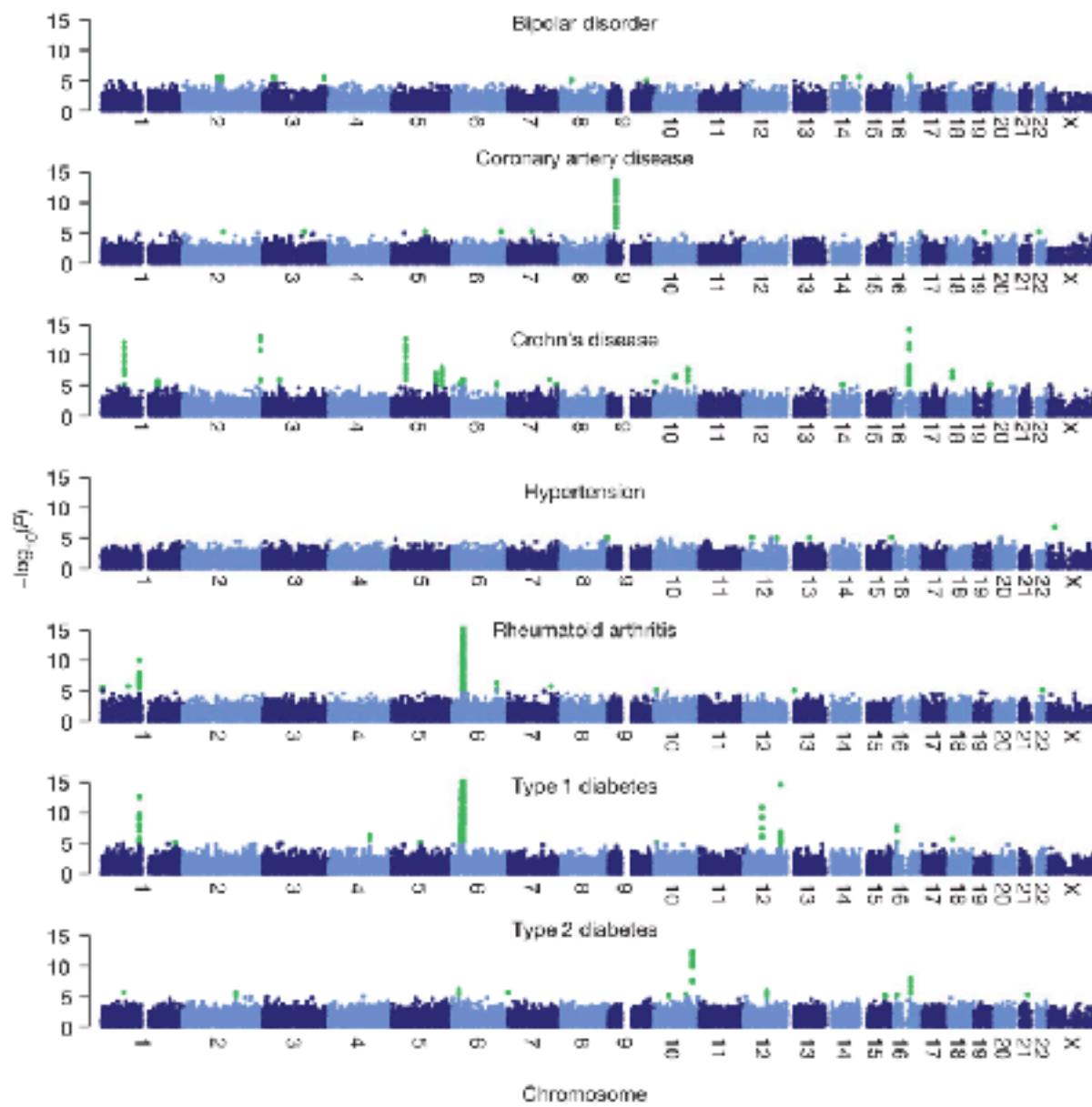
Aruna Helgadottir,¹ Gudmar Thorleifsson,¹ Andre Masselink,¹ Sigrun Grotundottir,¹ Unnur Thorsdottir,¹ Sigrun J. Sigurdardottir,¹ Guðrún Ólafsdóttir,¹ Steinn J. Gudjonsson,¹ Elinor M. Vidalineau,¹ Rögnvaldur E. Margeirsson,¹ Rúnar Johannesson,¹ Ólafur Ólafsson,¹ Marta Horwitz,² Jonathan A. Tager-Flusberg,² Margaret Thorleifsson,¹ Michael E. Fager,³ Eric J. Topper,³ Argentine Kong,⁴ Vilma Ólafsdóttir,¹ Ásthildur Hjartarsdóttir,¹ Jeffrey C. Dahlöf,⁵ Kari Stefansson¹

Helgadottir, A., et al. *Science* volume 316, 1491; 2007

Wellcome Trust Case-Control Consortium

- 1,500 1958 Birth Cohort Controls (58BC)
- 1,500 UK Blood Services Controls (UKBS)
- 14,000 cases of seven common diseases
 - Bipolar disorder
 - Coronary artery disease
 - Crohn's disease
 - Hypertension
 - Rheumatoid arthritis
 - Type 1 diabetes
 - Type 2 diabetes

The image shows a journal cover from Nature magazine. At the top left, it says "Vol 447 | 7 June 2007 doi:10.1038/nature05911". To the right is the "nature" logo. Below the title, the word "ARTICLES" is written in large, light-colored capital letters. A horizontal line separates the header from the main title. The main title of the article is "Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls". At the bottom, it credits "The Wellcome Trust Case Control Consortium".



One famous example

- deCODE Genetics was the first to discover a SNP associated with myocardial infarction (MI) in 2007
 - WTCCC, McPherson, and Samani were able to replicate the same finding in the same year, and many have reconfirmed it in different populations



A Common Variant on Chromosome 9p21 Affects the Risk of Myocardial Infarction

Aaro Halgadottir,^{1a} Guðmar Þorleifsson,^{1a} Andri Minsasco,^{1a} Sóleyv Grétar Þórdóttir,¹
 Þuríður Blöndu,¹ Áslaug Jónsdóttir,¹ Adalbjörg Jónsdóttir,¹ Ágæt Skarðsbjörn,¹
 Ás Ævar,¹ Ómar Ráðiðn,¹ Áslaug Úlfarsdóttir,¹ Daniel F. Gedjartsson,¹ Kristjan P. Magnússon,¹
 Karl Andersen,² Ásæn I. Leven,² Valgerður N. Þórmarsdóttir,¹ Sigrún Ólafsdóttir,¹
 Thorbjorg Jónsdóttir,¹ Stefán Þórhallsson,¹ Þórunn Gunnarsdóttir,¹
 Ásmundur Gylfason,¹ Viola Vacariu,³ W. Craig Hooper,³ Marianne P. Foley,⁴
 Christopher B. Gangar,¹ Farzam Austin,¹ David J. Reale,¹ Sveti H. Shah,⁵ Aristed A. Qayyum,⁵
 Jeffrey R. Gaither,¹ Guðmundur Þorgeirsson,² Úlfur Þorlindssóttir,¹
 Agustín A. Roca,¹ Karl Skulason,¹

A Common Allele on Chromosome 9 Associated with Coronary Heart Disease

Ruth McPherson,^{1,†} Alexander Fertsemidis,^{2*} Nihan Kavaslar,³ Alexandre Stewart,¹ Robert Roberts,¹ David R. Cox,³ David A. Hindi,³ Len A. Pennacchio,^{4,5} Anne Tybjærg-Hansen,¹ Aaron R. Folsom,⁷ Eric Boerwinkle,³ Heleen H. Hobbs,^{2,9} Jonathan C. Cohen^{2,10,†}

Helgadottir, A., et al. Science; 316(5830):1491-1493, 2007

McPherson, R., et al. *Science*; 316(5830):1488-1491, 2007

Wellcome Trust Case Control Consortium. *Nature*; 447(7145):661-678, 2007

Samani, N.J., et al. N Engl J Med; 357(5):443-453, 2007

The NEW ENGLAND
JOURNAL of MEDICINE

Genomewide Association Analysis of Coronary Artery Disease

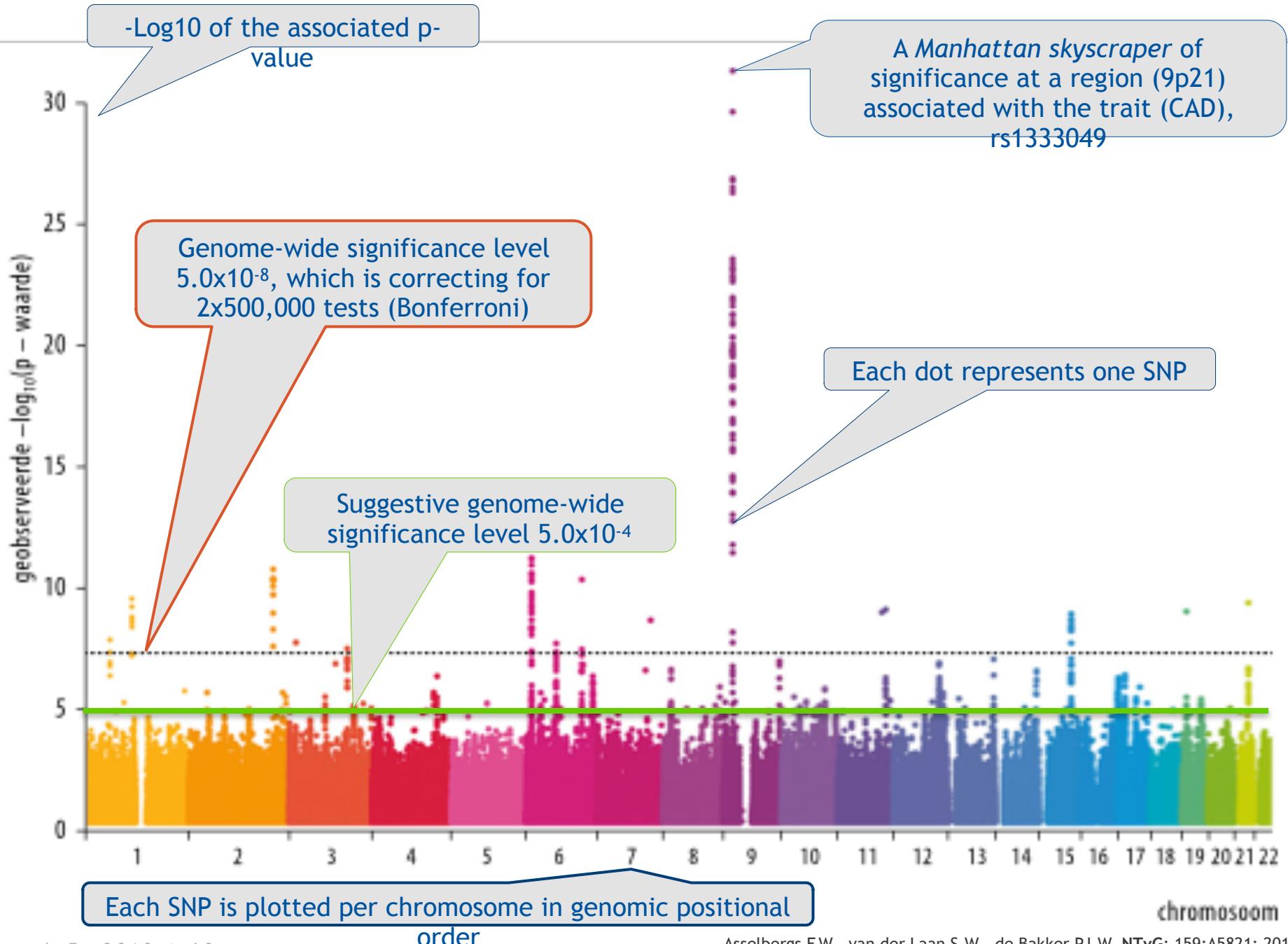
Ms. A.1.17/7 (verso) 2002.4.1120.1030/2014-05-20

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ARTICLES

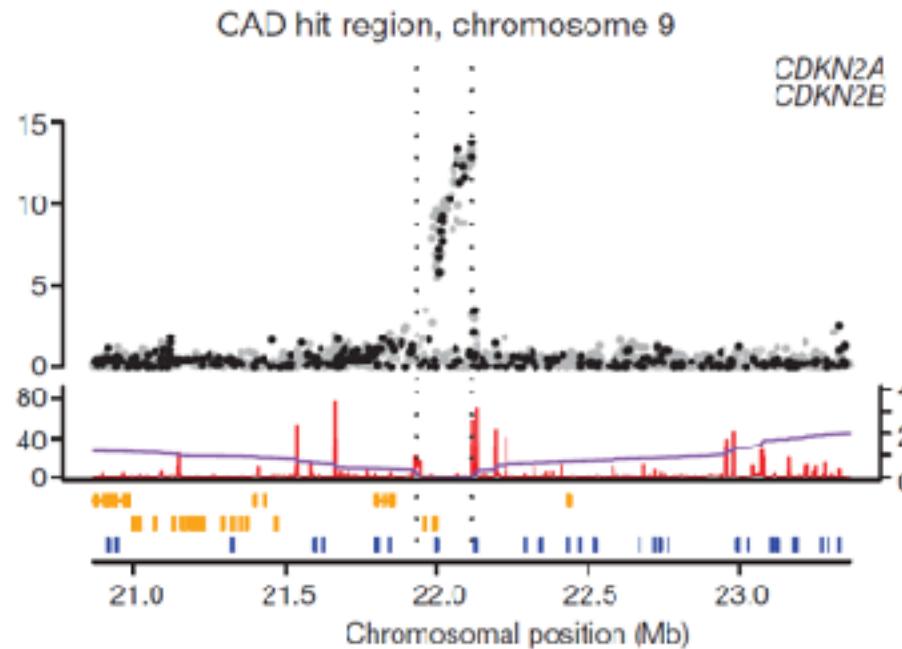
Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls

The Wellcome Trust Case Control Consortium



9p21 and cardiovascular disease

- The SNPs associated with CAD on 9p21.1 are rs1333049, rs10757274, rs2383207, rs2891168, and rs10757278
- They are found in an *intergenic region*
- Genes nearby: *CDKN2A*, *CDKN2B*
 - also associated with *type 2 diabetes mellitus*
 - regulating cell proliferation, cell aging and the associated degeneration, and programmed cell death of many cell types



Wellcome Trust Case Control Consortium. *Nature*; 447(7145):661-678, 2007

A closer look at the results...

Table 3 | Regions of the genome showing the strongest association signals

Collection	Chromosome	Region (Mb)	SNP	Trend P-value	Genotypic P-value	lnZ(BF), additive	lnZ(BF), general	Risk allele	Minor allele	Heterozygote odds ratio	Homozygote odds ratio	Control MAF	Case MAF
CAD	9p21	21.93-22.12	rs1333049	1.79×10^{-14}	1.16×10^{-13}	11.66	11.19	C	C	1.47 (1.27-1.70)	19 (161-2.24)	0.474	0.551

- CAD: coronary artery disease
- 9p21: chromosome 9, short arm (p)
- Region: 21.93-22.12 megabase pairs
- rs1333049: official dbSNP ID

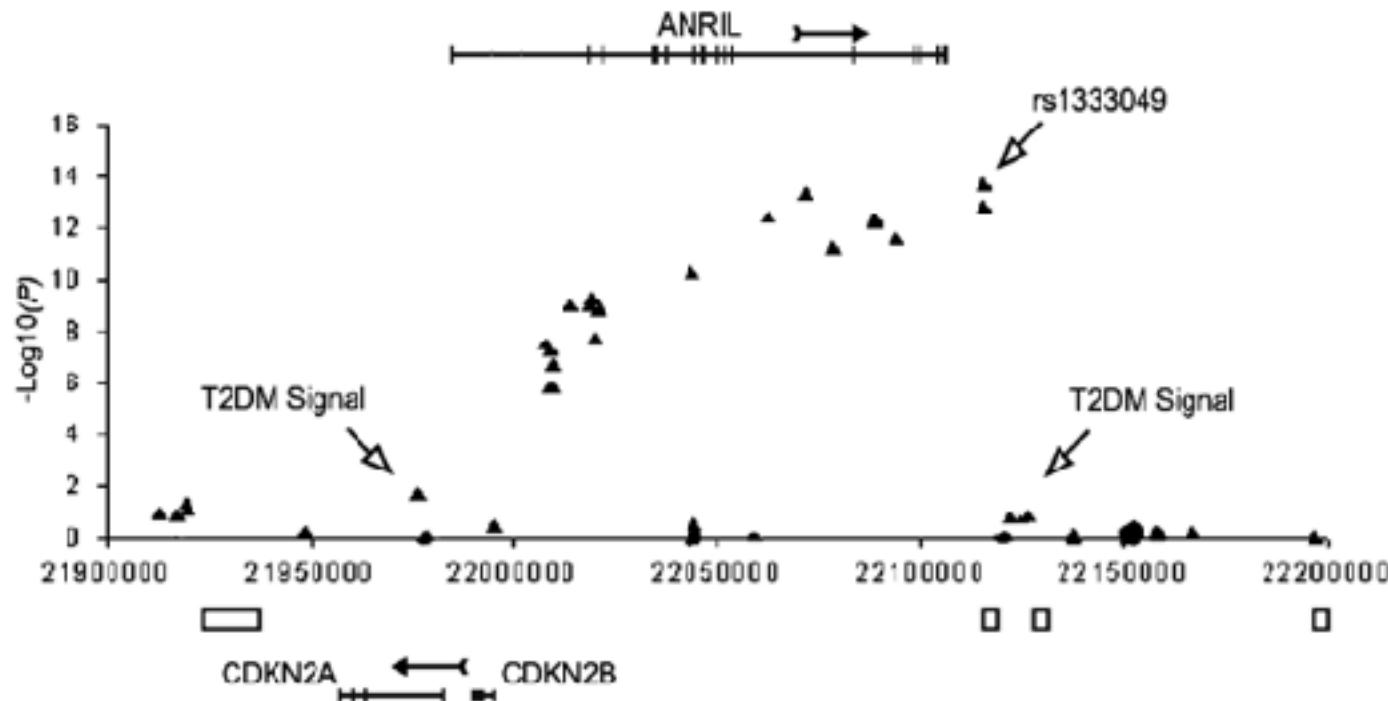
Risk allele: minor allele
 Odds ratio: the odds of exposure between cases and controls

P-value of association test: AA vs. AB vs. BB

Minor allele frequency: the frequency of the risk (minor) allele in the population

9p21 points to a RNA gene

- Resequencing unveiled a RNA gene, *ANRIL*
- Current efforts are aimed to elucidate the role of *ANRIL* in (A)MI
- Might be involved in *early-onset MI* (before age of 50 years)



Samani, NJ., et al. *Circ Cardiovasc Genet*; 1:81-84, 2008

CARDIoGRAMplusC4D Study

- Coronary Artery Disease Genome-Wide Replication And Meta-Analysis Study: CARDIoGRAM
- > 63,000 cases and > 130,000 controls
 - Myocardial infarction (MI), coronary artery disease (CAD) or both
 - CAD: MI, CABG, PTCA, AP
 - Age limit: 45-66
- Sample size greatly influences power and effect size to discover new variants
- CARDIoGRAMplusC4D sought to solves this issue
- 55 susceptibility loci for CAD were discovered



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

The CARDIoGRAMplusC4D Consortium¹

Coronary artery disease (CAD) is the commonest cause of death. Here, we report an association analysis involving 63,000 CAD cases and 130,661 controls identifying 55 lead, reaching genome-wide significance, taking the number of susceptibility loci for CAD to 46, and a further 144 independent variants ($p < 3.2 \times 10^{-8}$, strongly associated with CAD at a 1% false discovery rate (FDR). Together, these variants explain approximately 10% of CAD heritability. Of the 46 genome-wide significant lead loci, 2 show a significant association with a single trait, one shows a significant association with blood pressure, but none is significantly associated with diabetes. Network analysis with 281 candidate genes (lead at 1% FDR) generated 5 interaction networks comprising 85% of these pathway partners involved in CAD. The less-met significant pathways mapping to these networks are linked to lipid metabolism and inflammation, underscoring the causal role of these activities in the genetic etiology of CAD. Our study provides insight into the genetic basis of CAD and identifies key biological pathways.

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Coronary artery disease (CAD) is the commonest cause of death. Although, epidemiological studies have identified many risk factors for CAD, including plasma lipid concentration, blood pressure, smoking, diabetes and markers of inflammation, a causal link has been proven only for some, for example, low-density lipoprotein (LDL) cholesterol and blood vessels, primarily through randomized controlled drug therapy directed at the risk factor². Twin and family studies have documented the significant heritability of CAD^{3–5}. However, the heritability of CAD is low (only 1% to 10%, $p < 0.05$)^{6,7}. Because power per unit of heritability is proportional to sample size, genome-wide association analysis has the potential to define which risk factors are indeed causative to identify pathways and therapeutic targets⁸. To date, genome-wide association studies (GWAS) have successfully reported a total of 31 leads associated with CAD risk, significant with significance ($p < 5 \times 10^{-8}$)^{9–11. However, variants at these loci explain at least 10% of the heritability of CAD. One likely reason for this is given, given the polygenic nature of complex traits and the relatively small otherwise often sizes of the loci identified, many genuinely associated variants fail to reach the stringent FDR threshold of genome-wide significance. Indeed, there is increasing evidence that the gene is an arbitrary chromosome tract involving a large number of causative alleles with very small effects¹². Addressing this will require the discovery of additional lead variants leveraging large-scale genomic data to identify the molecular pathways underlying the pathogenesis of CAD. Such discovery is facilitated by building molecular networks, on the basis of RNA, DNA and protein interactions, which have robust inherent biological function that also show evidence of association with risk variants for CAD and related metabolic traits.}

In the largest GWAS meta-analysis of CAD, undertaken to date by the Coronary Artery Disease Genome-wide Replication and

Screening (CARDIoGRAM) Consortium¹, which involved 12,731 cases and 16,172 controls, identified 10 genome-wide significant associations, a linkage disequilibrium (LD)-based set of 6221 variants achieved a nominally significant P value of less than 3.0E-8. Here, we include 6223 SNPs in a meta-analysis involving 190,320 individuals, with the primary aim of defining additional susceptibility loci for CAD. In this work, we used the Metabochip array¹³, which consists of 687 FST SNPs (linkage disequilibrium (LD)-based set) and 6221 nominally significant SNPs, to identify associations in several additional cohorts (HIS, including CAD, and 100 flow map) conditioned for these traits. All SNPs in the array with data in the CARDIoGRAM study were considered for analysis (27,138 SNPs), of which 6,221 were the replicated SNP and 10,707 were fine-mapping SNPs in the 22 CAD susceptibility loci identified at the time at which the array was designed. The remaining 3378 were selected by the other consortia contributing the Metabochip array.¹³ In addition, we assess whether the genome-wide significant CAD risk variants act through traditional risk factors by considering the available large GWAS for diseases^{14–16}. Finally, we identify a broader set of SNPs passing a conservative FDR threshold for association with CAD and use this set to undertake network analysis to find key biological pathways underlying the pathogenesis of CAD.

RESULTS

Study design

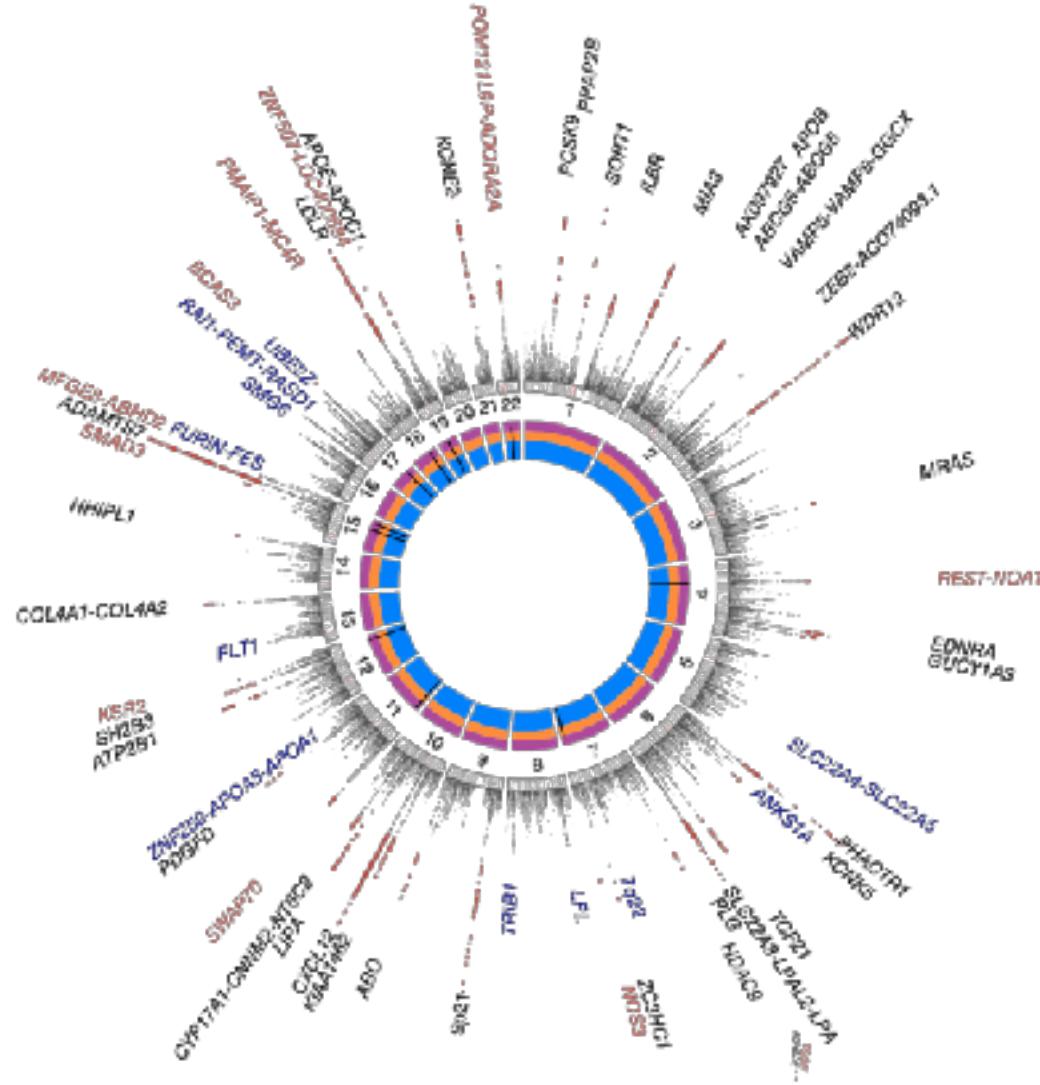
We analyzed the CARDIoGRAM discovery dataset (21,133 cases and 64,762 controls; stage I) with 34 additional CAD sample collections (stage II) (European or south Asian descent; comprising 61,310 cases and 65,955 controls) (study descriptions and sample characteristics are given in Supplementary Tables 1 and 2); results from all individuals from a 2-stage meta-analysis (n = 155,955) of the Metabochip array

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Received 24 April; accepted 29 November; published online 2 December 2012; doi:10.1038/ng.2840

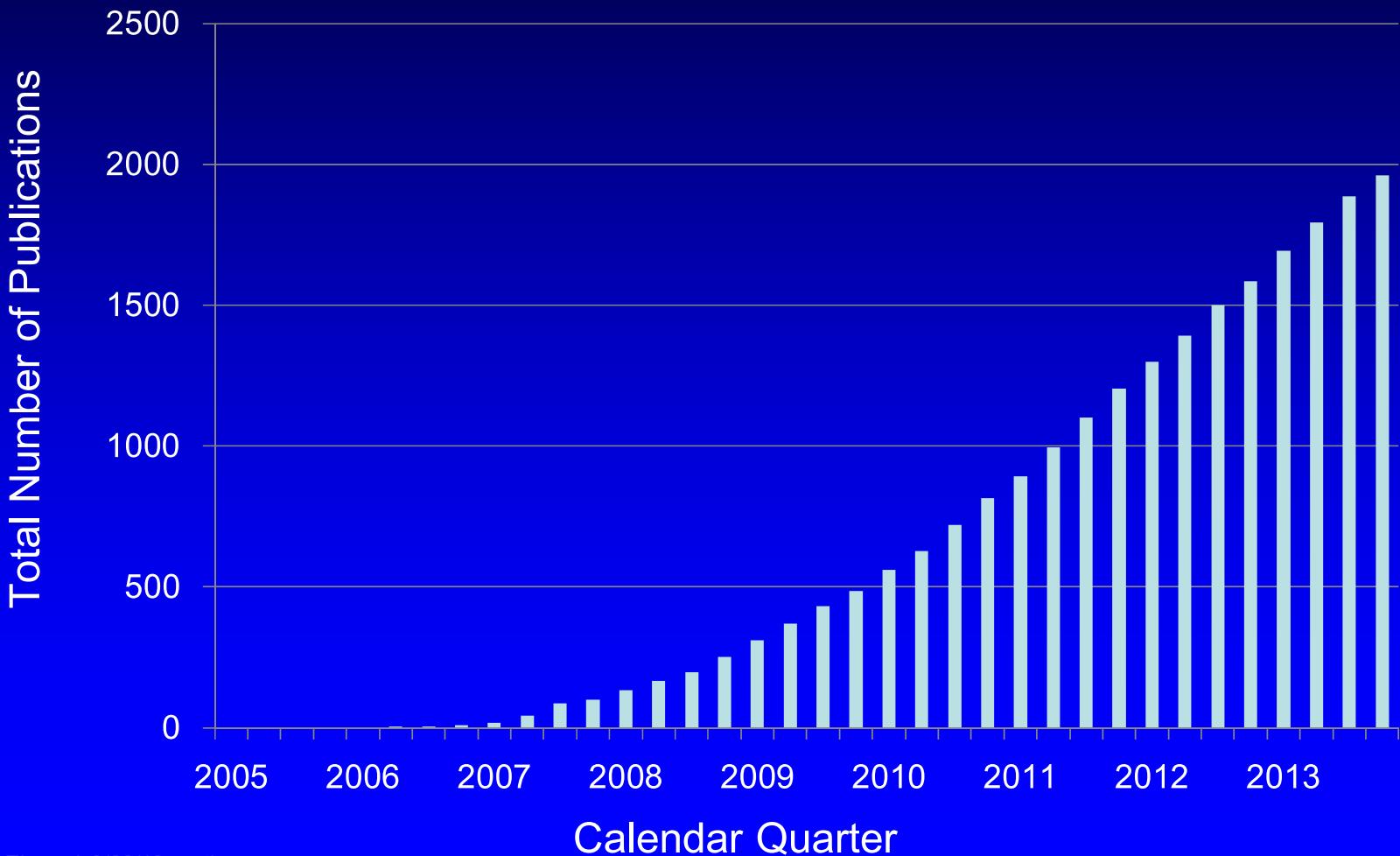
NATURE GENETICS | VOLUME 44 | NUMBER 1 | JANUARY 2012

And 8 years later (>15 times more samples)

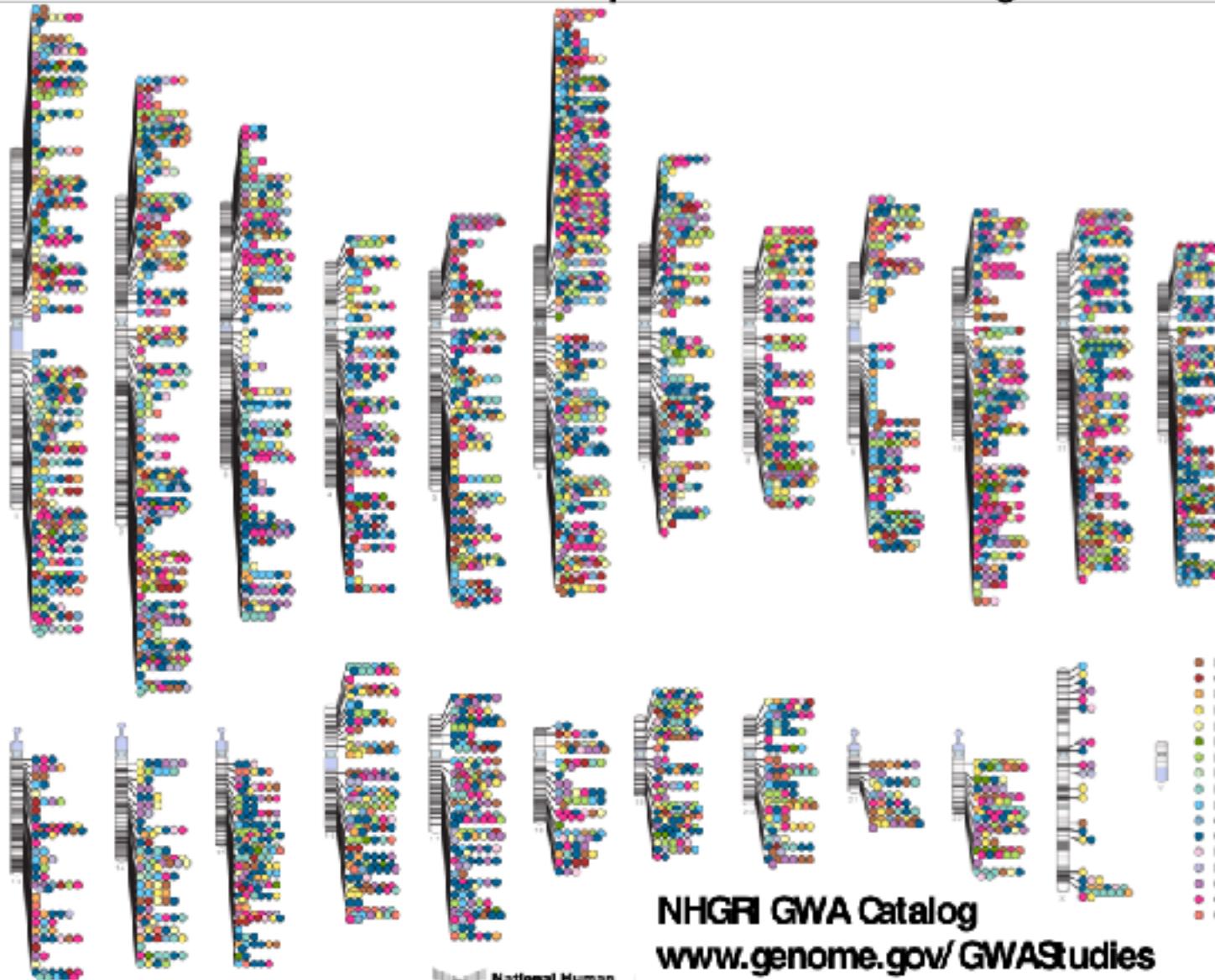


Published GWA Reports, 2005 – 2013

1960



Published Genome-Wide Associationst through 12/2013
Published GWA at $p \leq 5 \times 10^{-8}$ for 17 trait categories



NHGRI GWA Catalog
www.genome.gov/GWASStudies
www.ebi.ac.uk/fgpt/gwas/



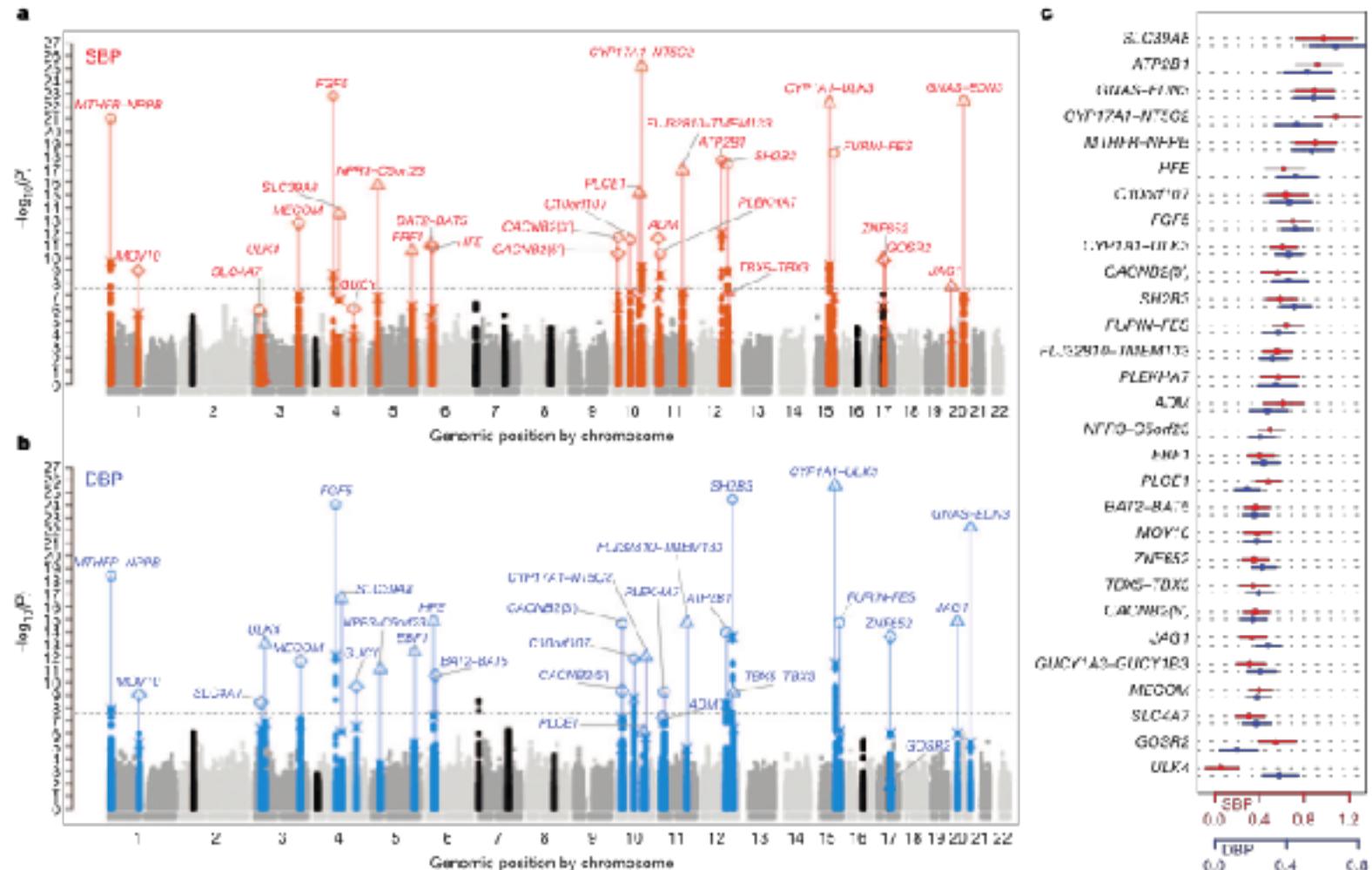


Figure 1 Genome-wide $-\log_{10} P$ -value plots and effects for significant loci. **a, b**, Genome-wide $-\log_{10} P$ -value plots are shown for SBP (a) and DBP (b). SNPs within loci reaching genome-wide significance are labelled in red for SBP and blue for DBP (± 2.5 Mb of lowest P value) and lowest P values in the initial genome-wide analysis as well as the results of analysis including validation data are labelled separately. The lowest P value in the initial GWAS are denoted with a X. The range of different sample sizes in the final meta-

analysis including the validation data are indicated as: circle (96,000–140,000), triangle ($> 140,000$ –180,000) and diamond ($> 180,000$ –220,000). SNPs near unconfirmed loci are in black. The horizontal dotted line is $P = 2.5 \times 10^{-8}$. GUCY denotes GUCY1A3, GUCY1B3. **c**, Effect size estimates and 95% confidence bars per blood-pressure-increasing allele of the 29 significant variants for SBP (red) and DBP (blue). Effect sizes are expressed in mm Hg per allele.

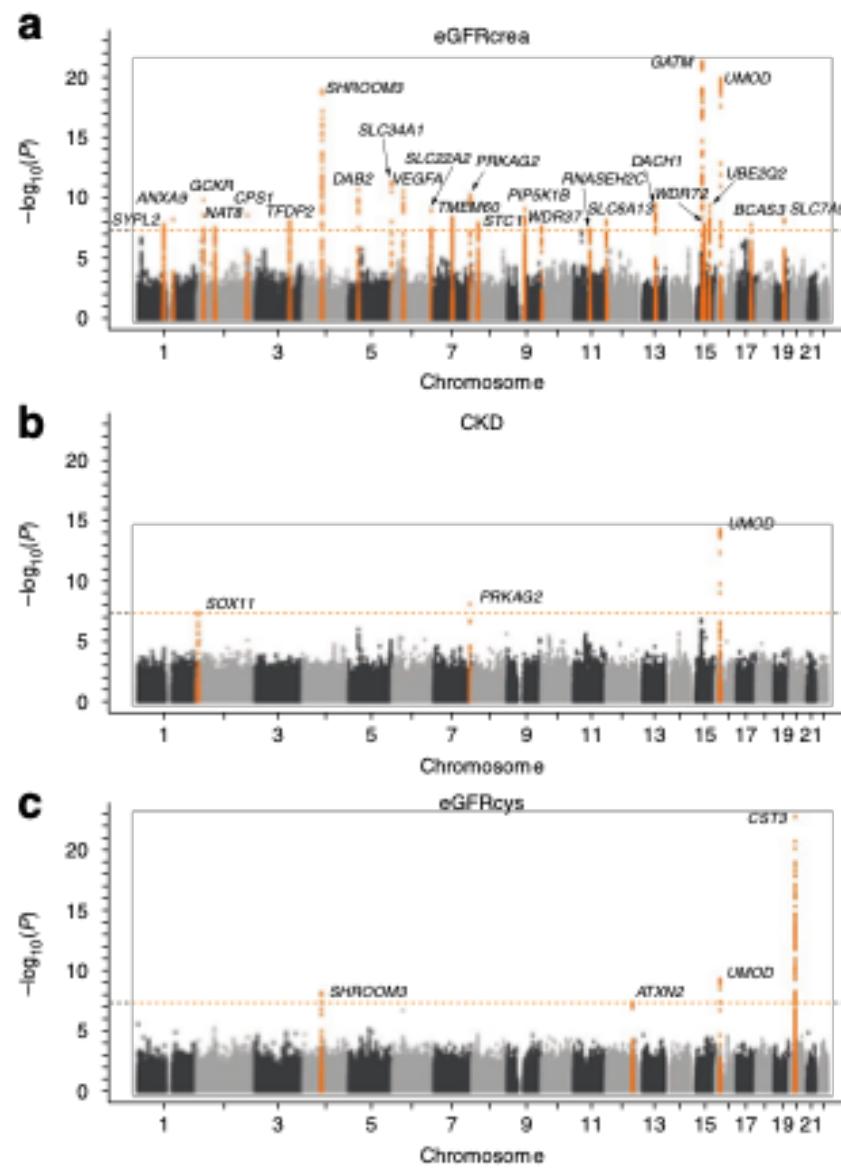


Figure 1 Genome-wide $-\log_{10} P$ value plot from stage 1. (a–c) Plots show discovery analysis of eGFRcrea (a), CKD (b) and eGFRcys (c). The dotted line indicates the genome-wide significance threshold at $P = 5 \times 10^{-8}$.

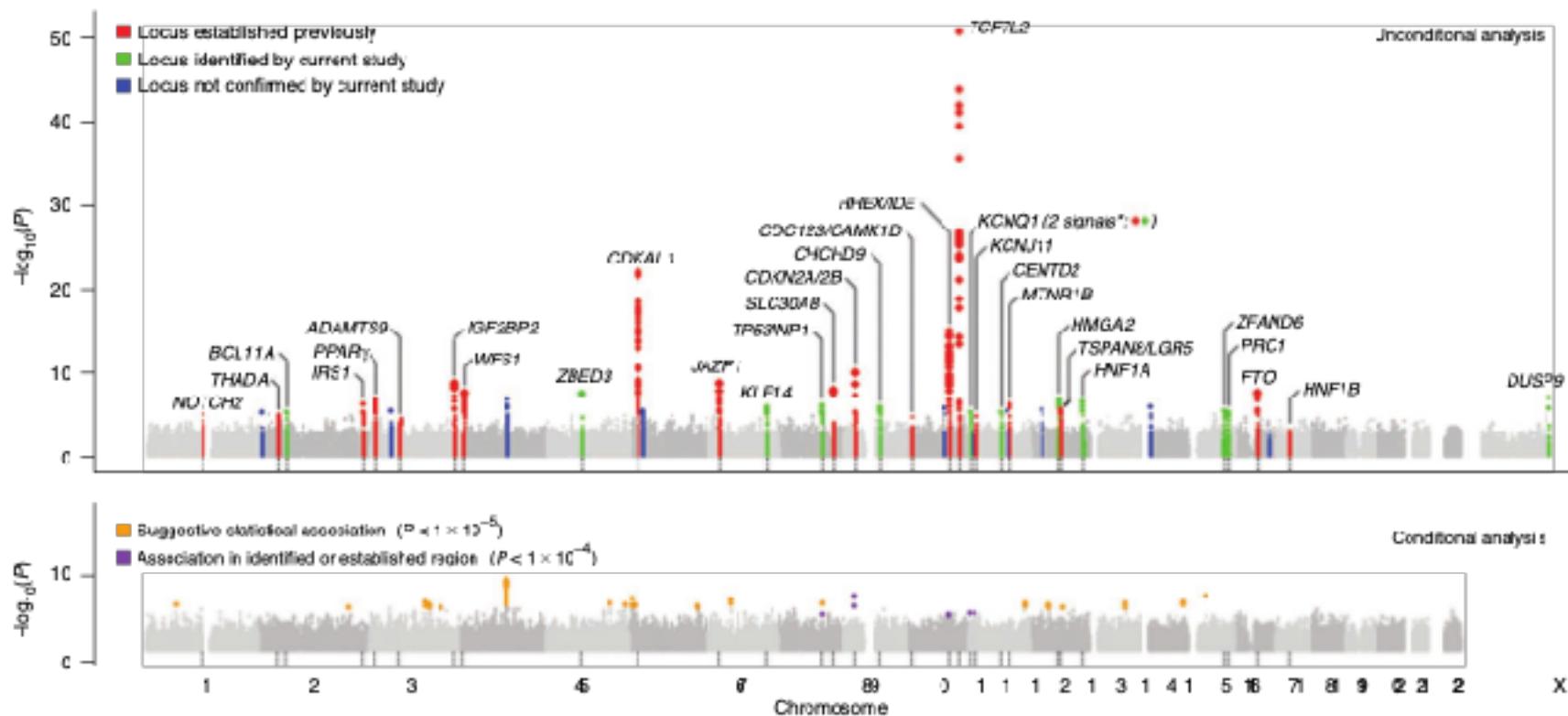
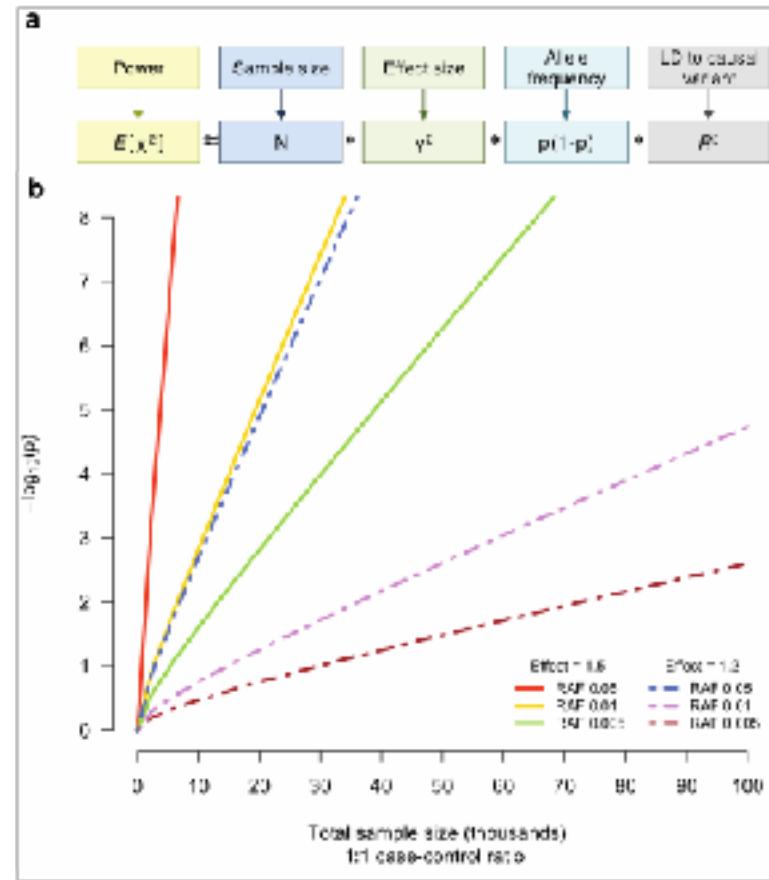
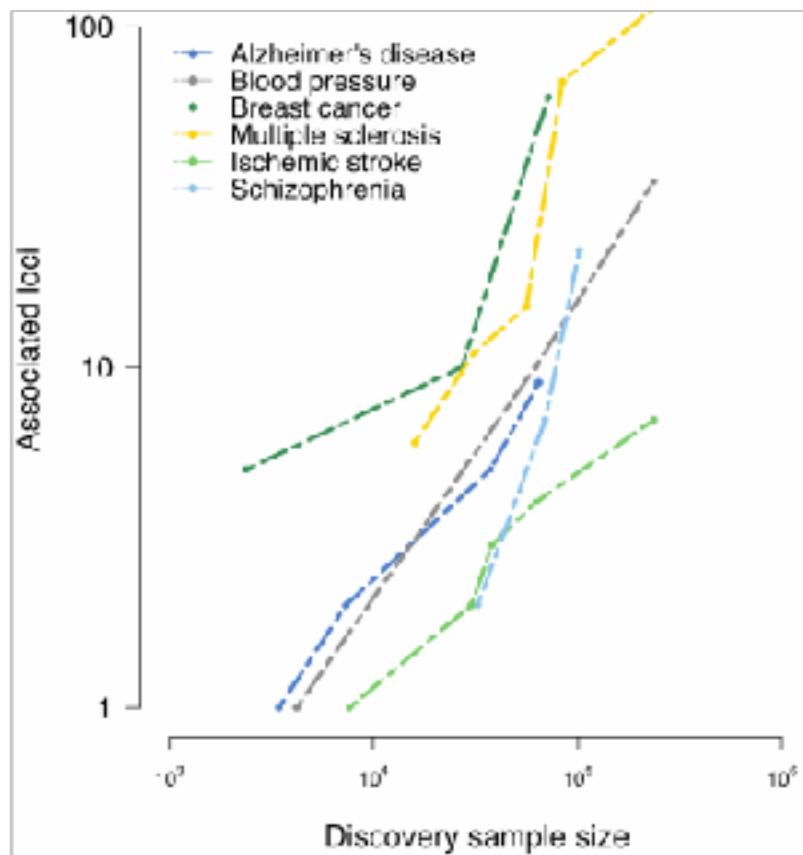
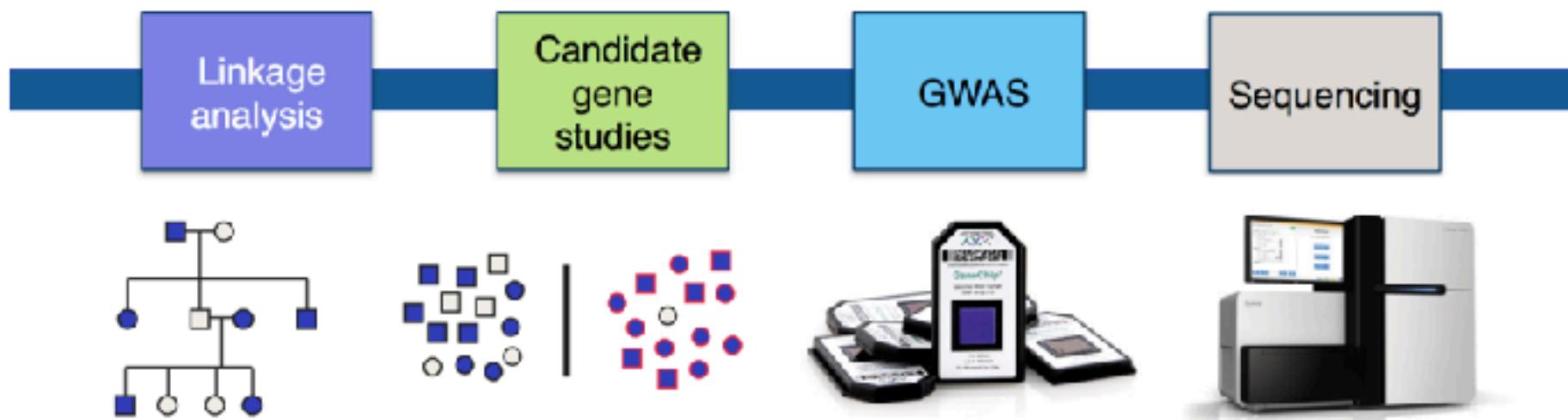
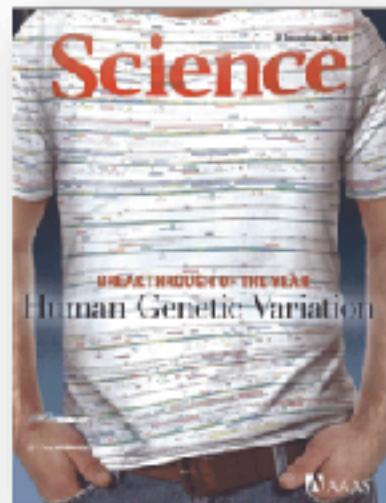
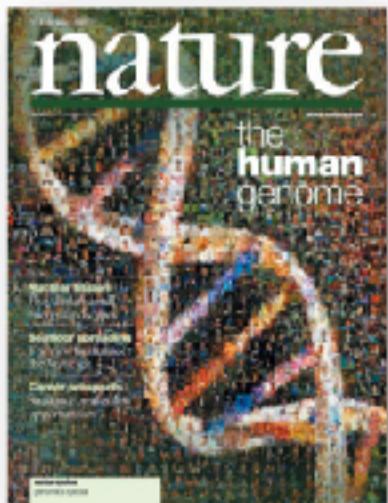


Figure 1 Genome-wide Manhattan plots for the DIAGRAM+ stage 1 meta-analysis. Top panel summarizes the results of the unconditional meta-analysis. Previously established loci are denoted in red and loci identified by the current study are denoted in green. The ten signals in blue are those taken forward but not confirmed in stage 2 analyses. The genes used to name signals have been chosen on the basis of proximity to the index SNP and should not be presumed to indicate causality. The lower panel summarizes the results of equivalent meta-analysis after conditioning on 30 previously established and newly identified autosomal T2D-associated SNPs (denoted by the dotted lines below these loci in the upper panel). Newly discovered conditional signals (outside established loci) are denoted with an orange dot if they show suggestive levels of significance ($P < 10^{-5}$), whereas secondary signals close to already confirmed T2D loci are shown in purple ($P < 10^{-4}$).

Power, Effect size, Sample size...

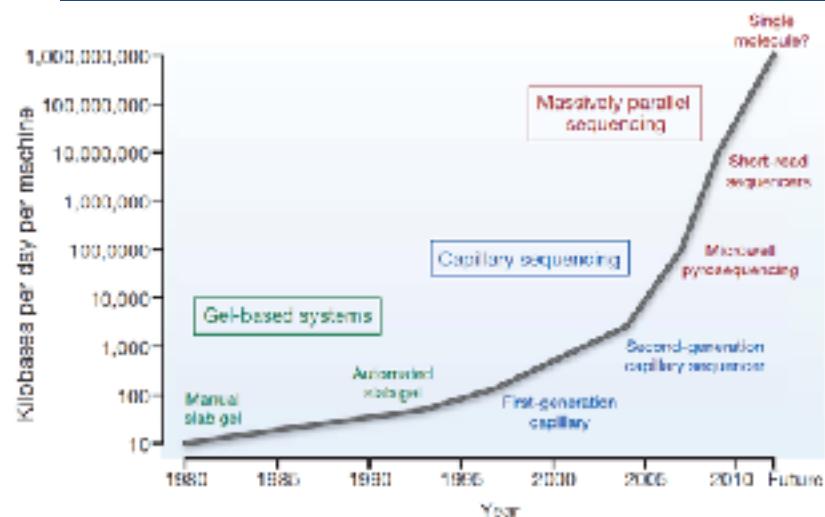
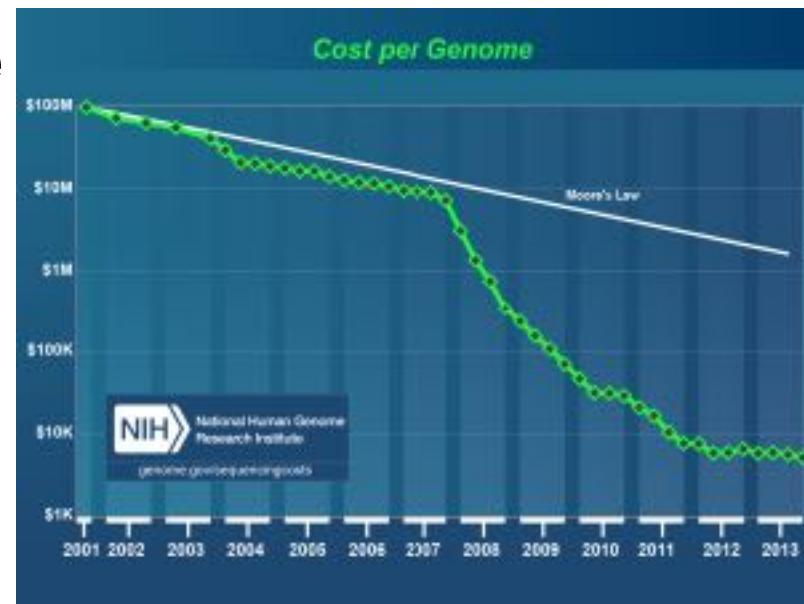




Next-generation sequencing

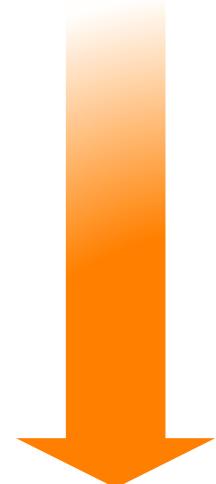
Milestone: \$1000 dollar genome
(2014, Illumina HiSeq X Ten Sequencer)

But how much money needs to be spent on annotation and (even more important) interpretation of the results?

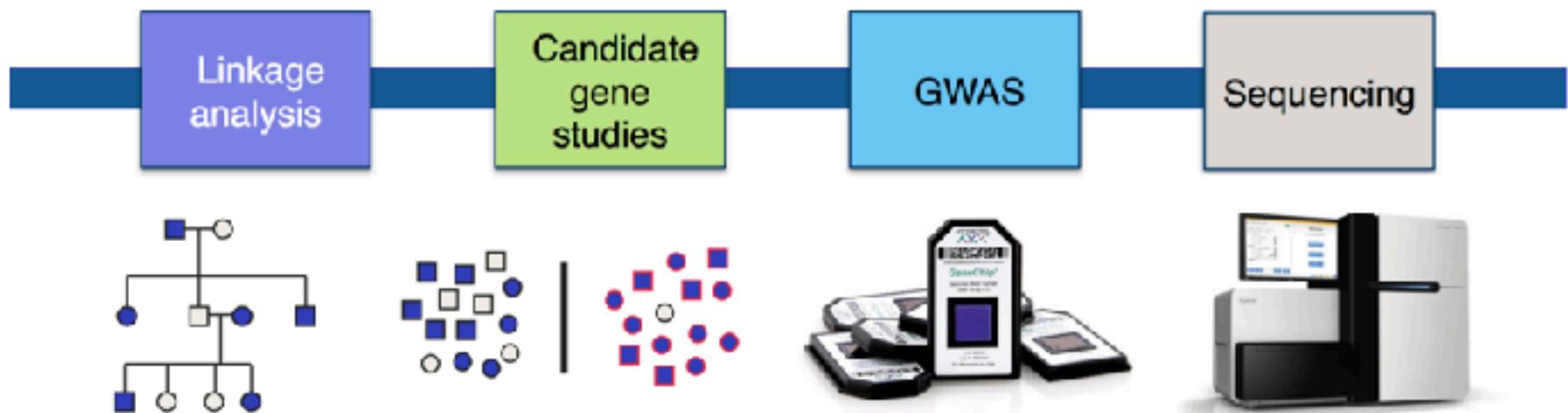


Summary: what's been (being) done?

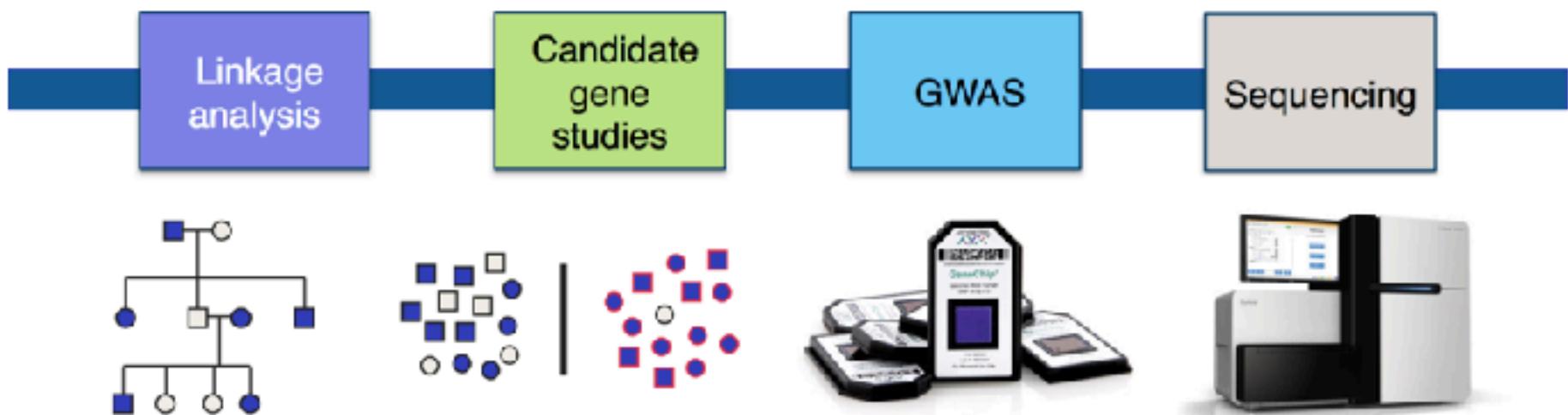
- Family-based linkage studies
 - Rare, Mendelian traits
- Candidate gene association studies
 - Many claims, few robust findings
 - Terrible track record in terms of reproducibility
- Genome-wide association studies (GWAS)
 - Complex traits and common diseases
- Whole-exome sequencing studies
 - Rare, Mendelian diseases (unsolved cases)
 - Complex traits and common diseases
- Whole-genome sequencing studies



What have we learned in the field of cardiovascular genetics?



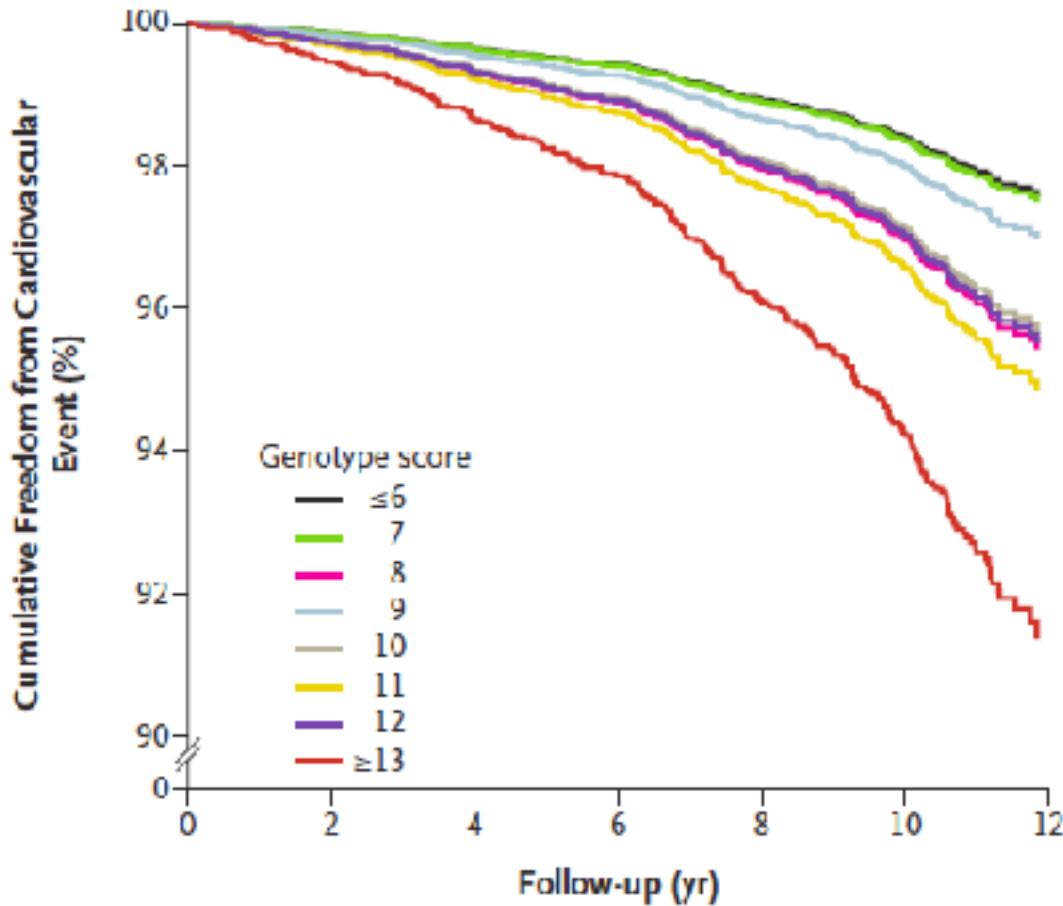
Prediction?



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,		<0.0001†
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

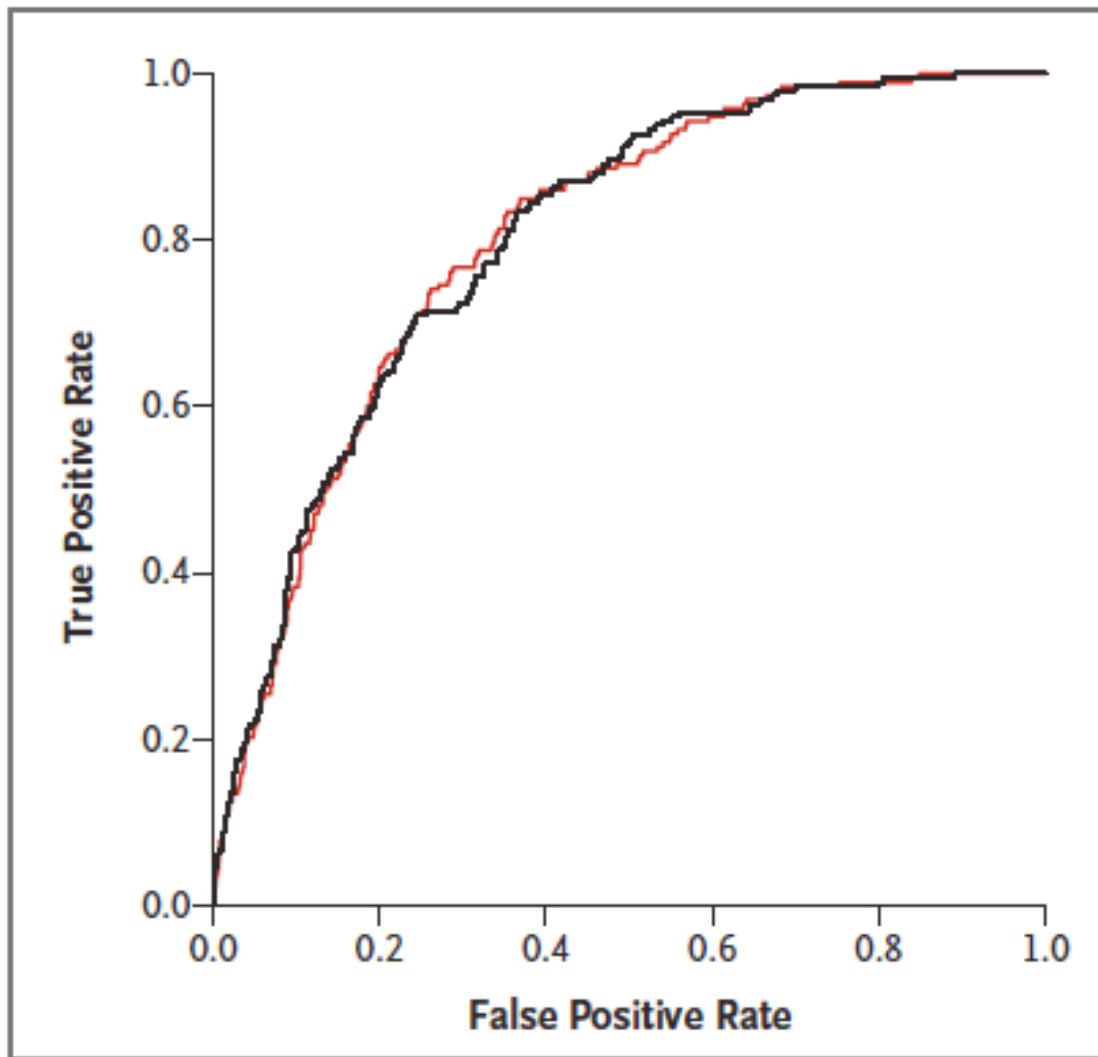


Figure 2. Receiver-Operating-Characteristic (ROC) Curves for Incident Myocardial Infarction, Ischemic Stroke, or Death from Coronary Heart Disease during 10-Year Follow-up.

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 ▾

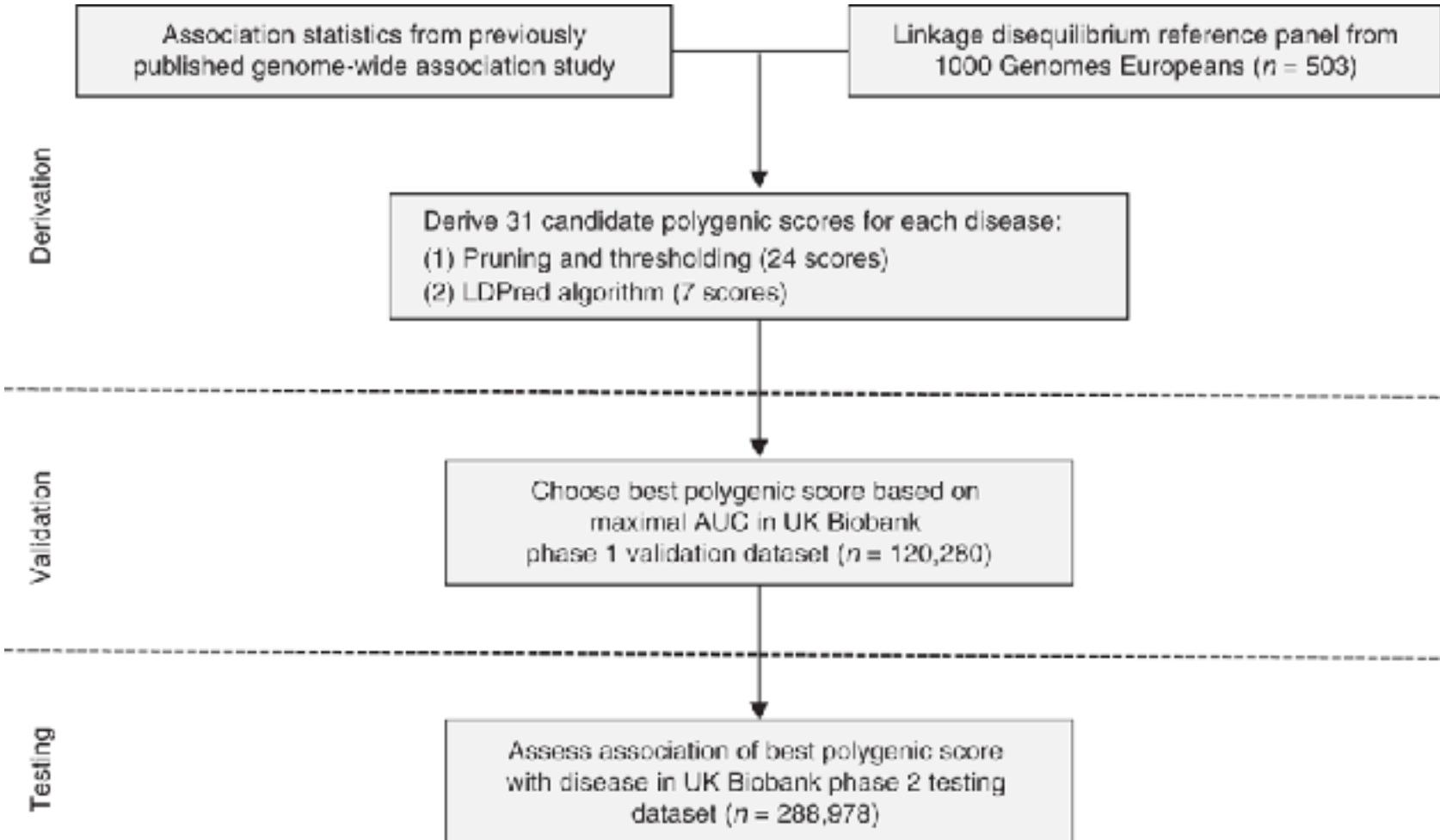
Letter | Published: 13 August 2018

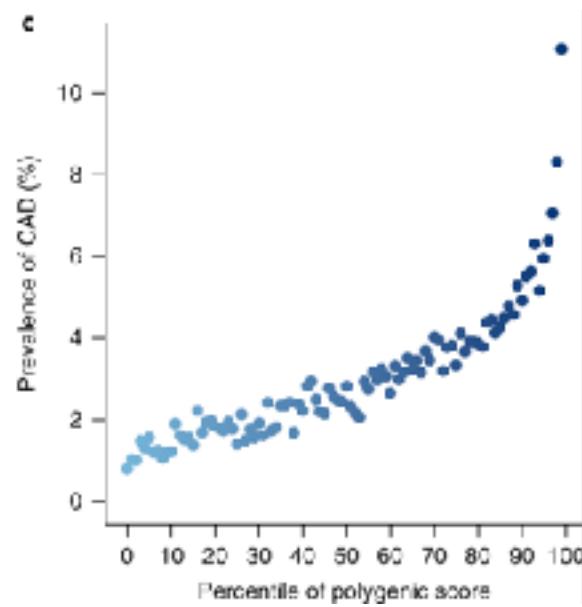
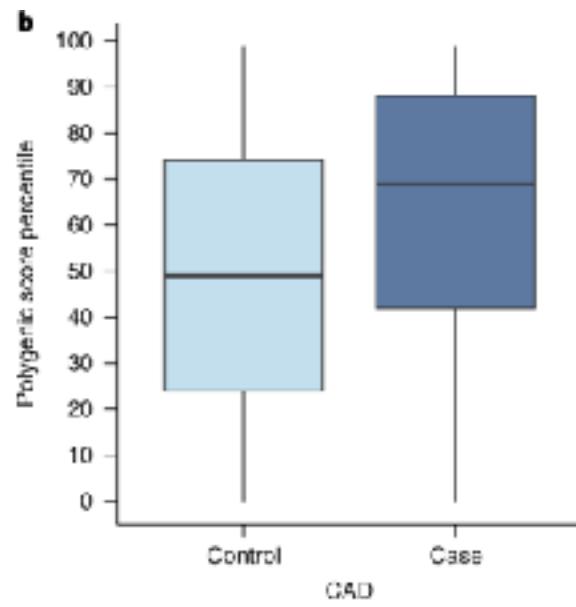
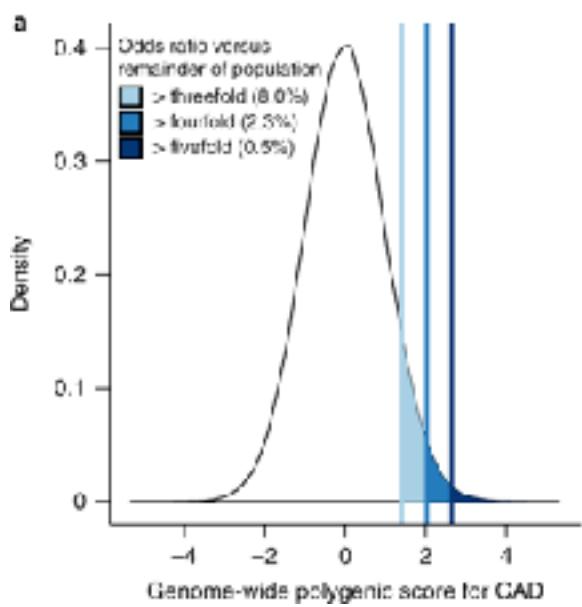
Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations

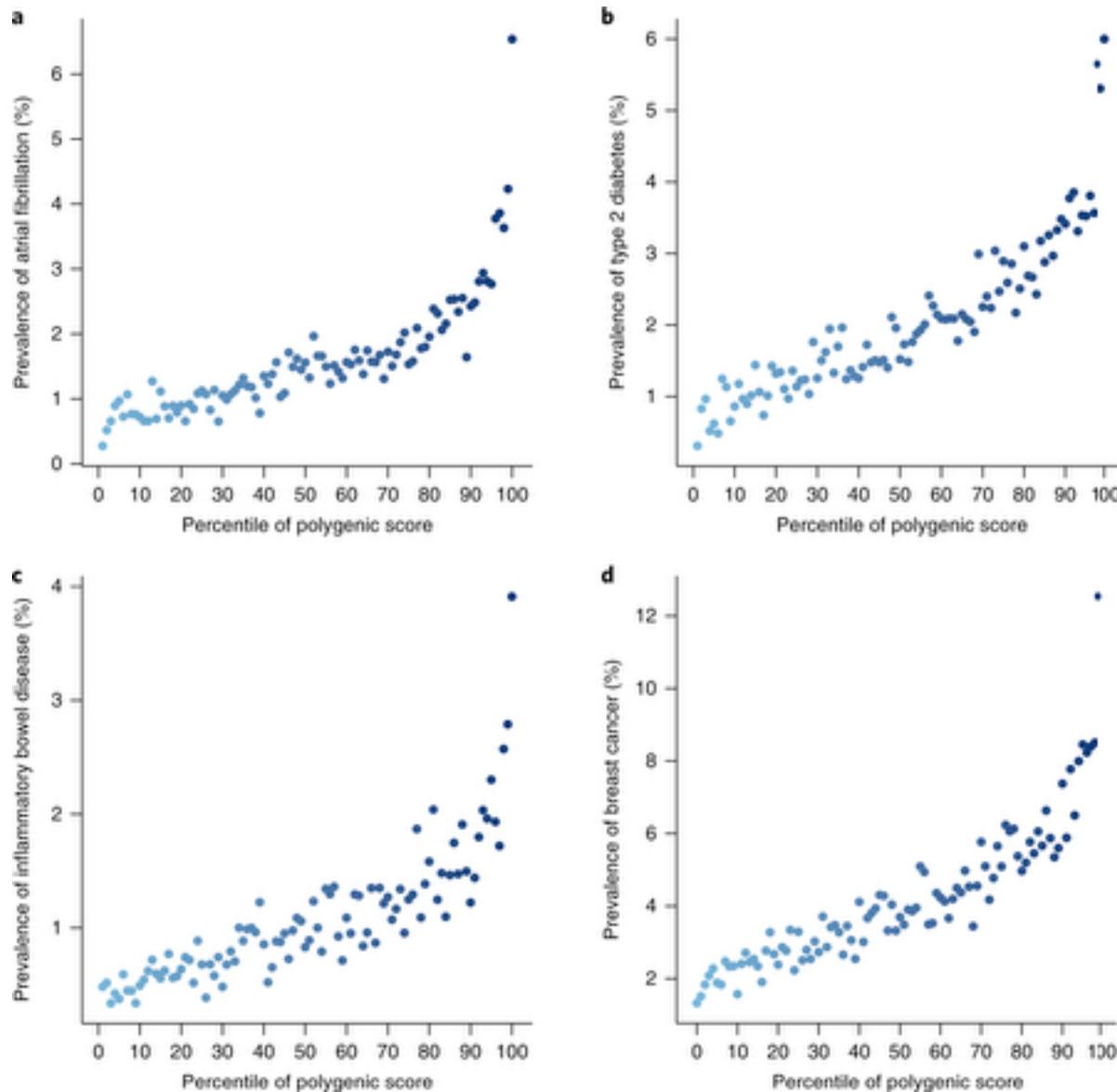
Amit V. Khera, Mark Chaffin, Krishna G. Aragam, Mary E. Haas, Carolina Roselli, Seung Hoan Choi, Pradeep Natarajan, Eric S. Lander, Steven A. Lubitz, Patrick T. Ellinor & Sekar Kathiresan ✉

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

Include millions of variants of small effects







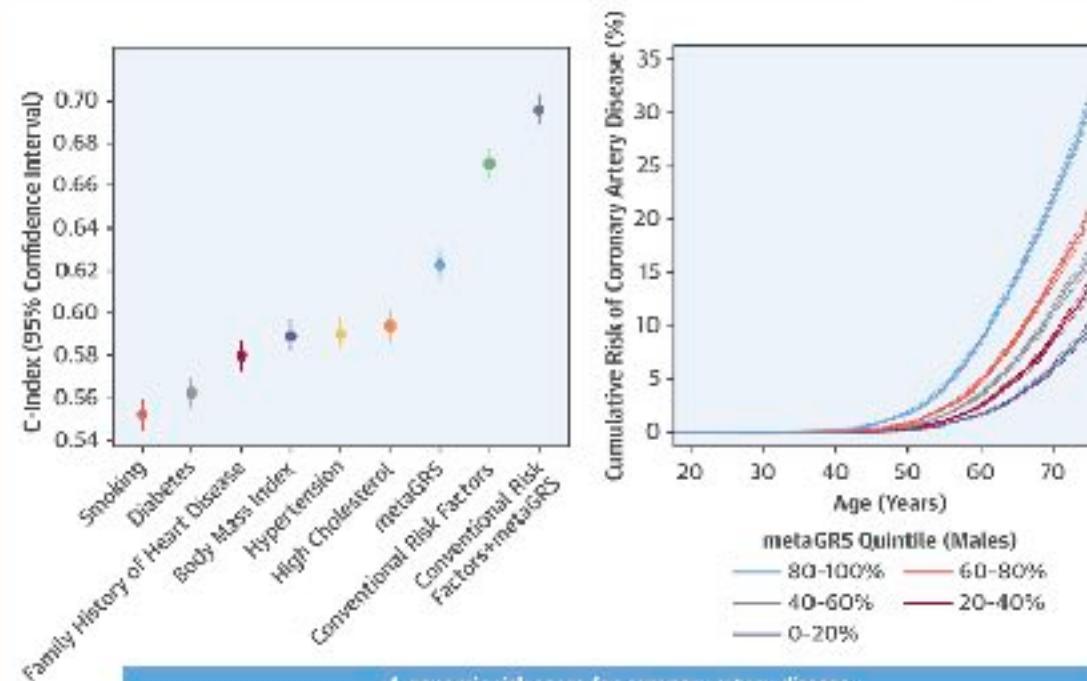
High GPS definition	Individuals in testing dataset (n)	% of individuals
Odds ratio ≥3.0		
CAD	23,119/288,978	8.0
Atrial fibrillation	17,627/288,978	6.1
Type 2 diabetes	10,099/288,978	3.5
Inflammatory bowel disease	9,209/288,978	3.2
Breast cancer	2,369/157,895	1.5
Any of the five diseases	57,115/288,978	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 450,000 Adults

Implications for Primary Prevention

Michael Inouye, Gadi Abramson, Christopher P. Nelson, Angela M. Wong, Michael J. Sweeting, Hank Dartbridge, Florence Y. Liu, Stephen Kapur, Maria Brancisca, Thanting Wang, Shu Ye, Thomas F. Pischl, Martin K. Runter, Joanna Tzoulaki, Riyad S. Patel, Ruth J.F. Lowe, Bernard Keavney, Harry Hensingway, John Thompson, Hugh Watkins, Panos Deloukas, Evangelos D. Angelantonio, Adam E. Butterworth, John Danesh, Niles J. Samani and for the UK Biobank CardioMetabolic Consortium CGR Working Group

CENTRAL ILLUSTRATION: Genomic Risk Score for Coronary Artery Disease

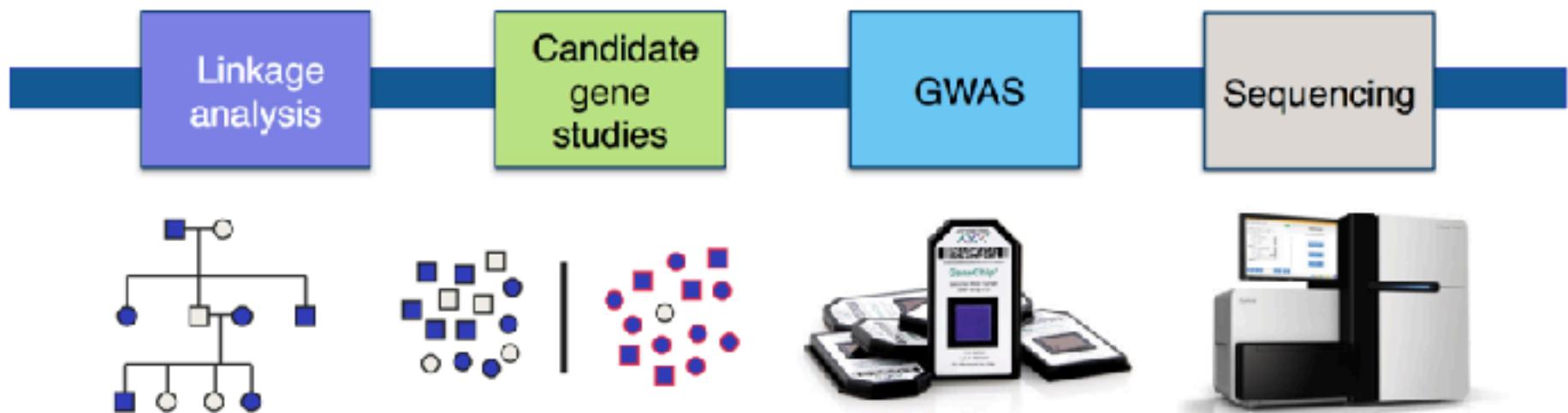


A genomic risk score for coronary artery disease

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.

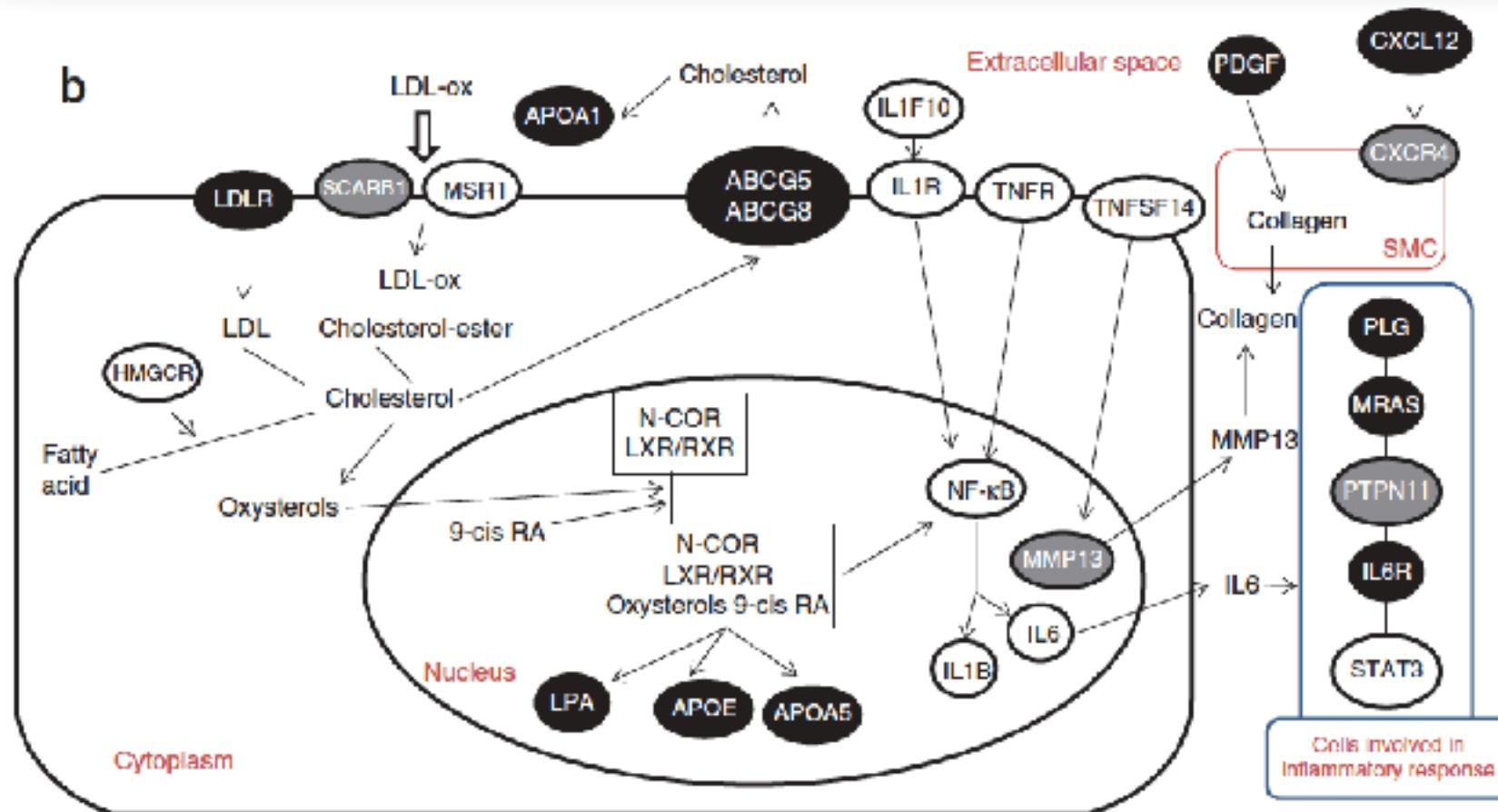
Biological mechanisms?



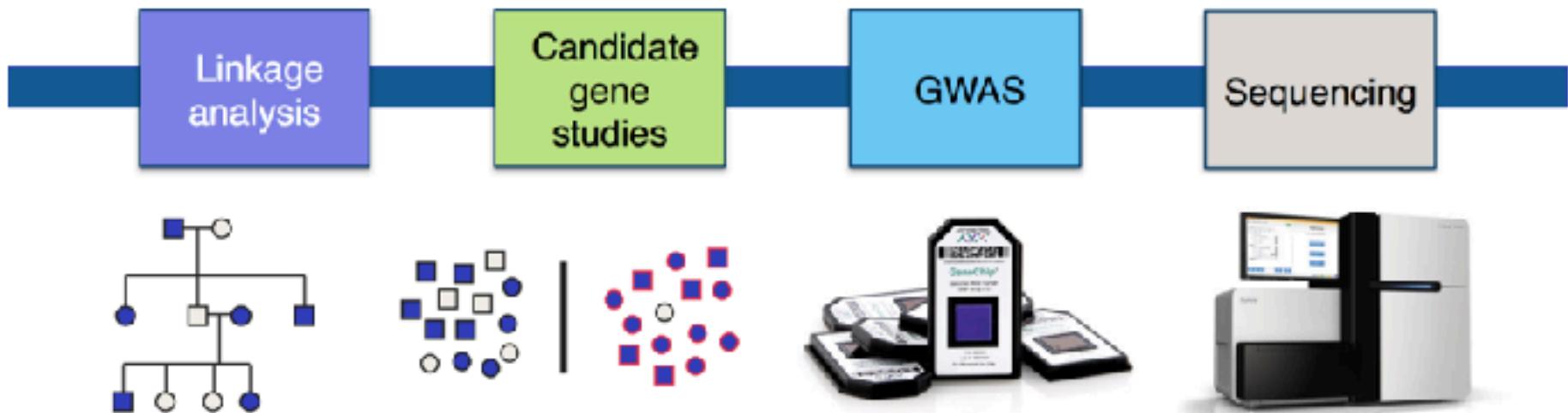
Large-scale association analysis identifies new risk loci for coronary artery disease

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. *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, 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.
7. Robertson FW, Cumming AM. Effects of Apolipoprotein E Polymorphisms on Serum Lipoprotein Concentration. *Atherosclerosis* 1985; 5: 283-92.
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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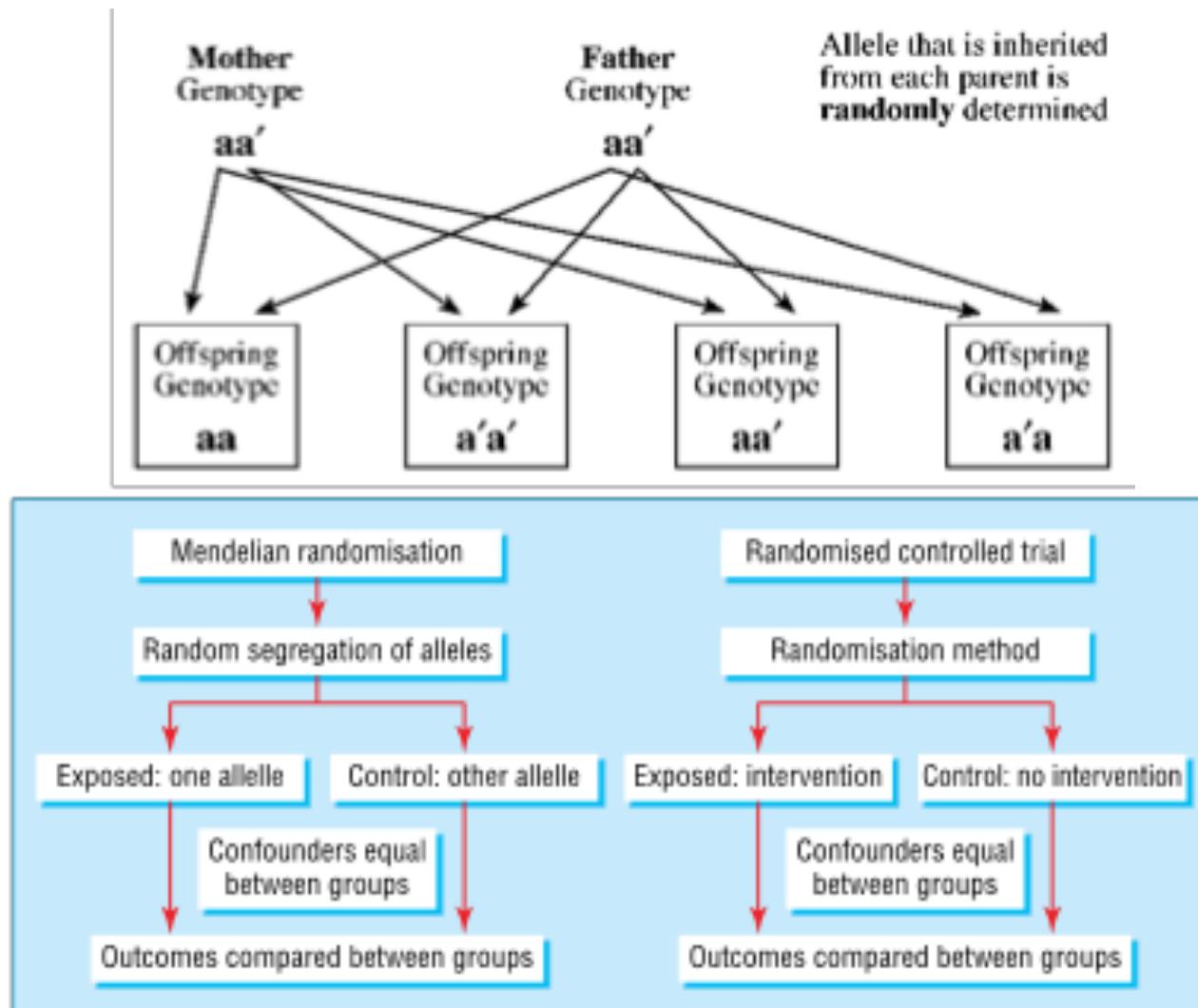
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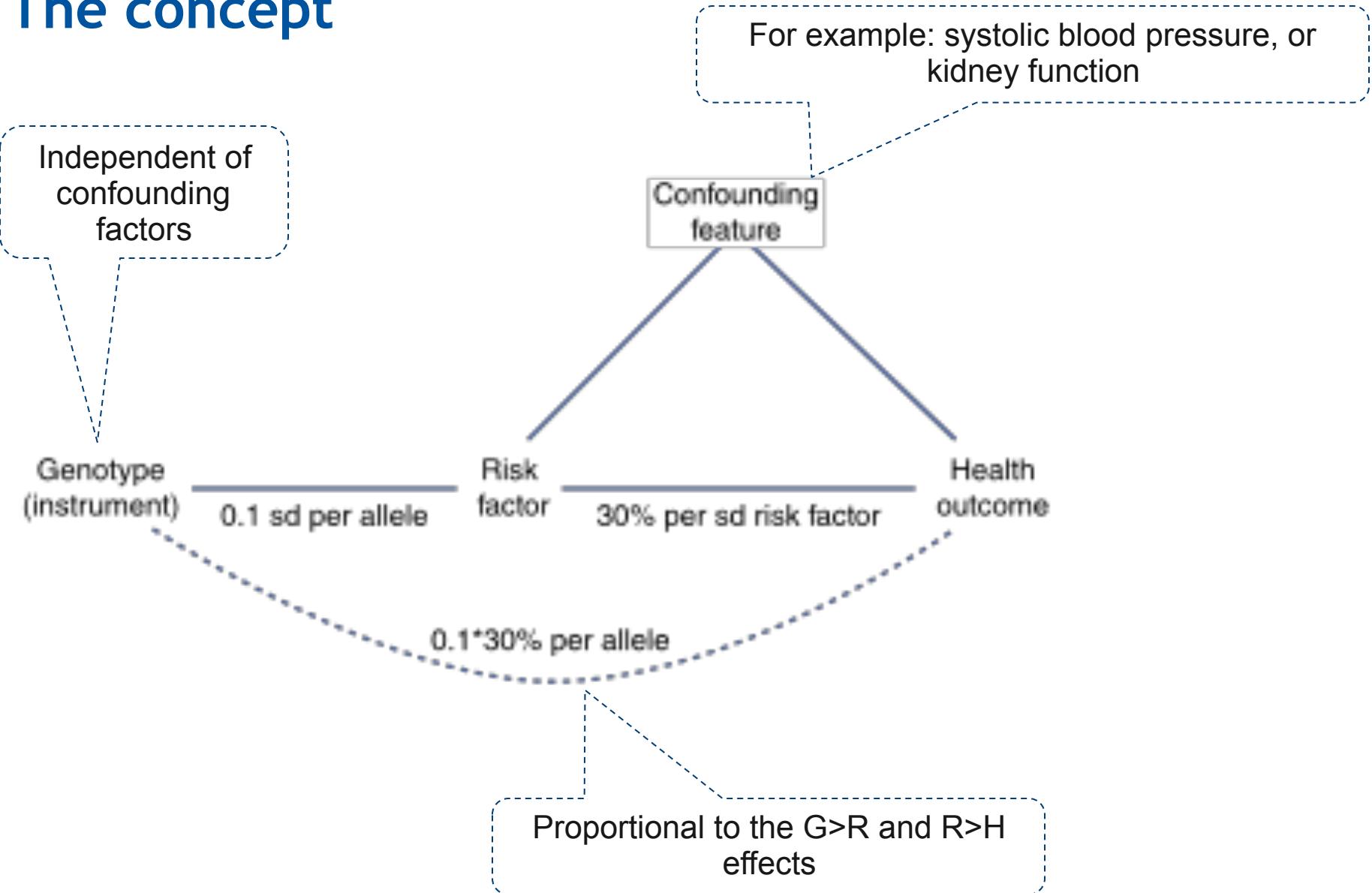


Gregor Johann Mendel - 20 July 1822 – 6 January 1884

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



The concept



Hypothesis: Inhibition of CETP might lead to raised HDL

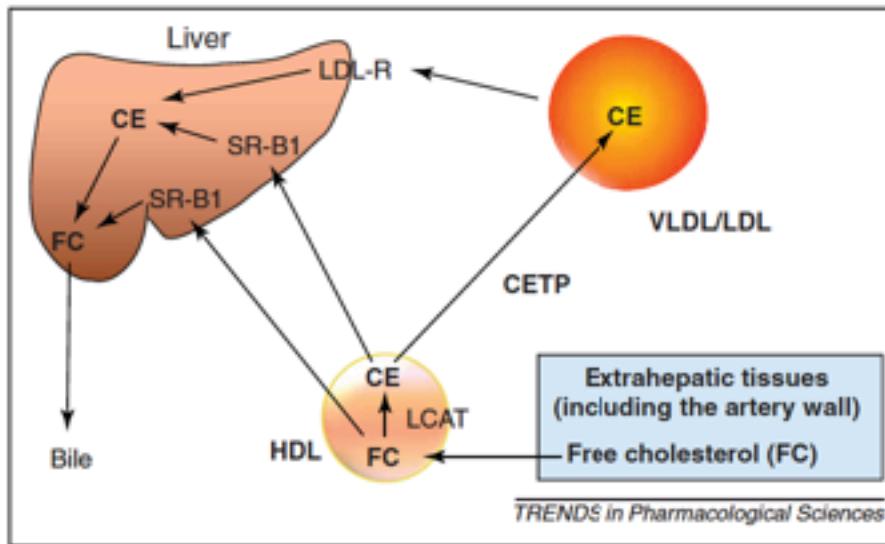


Figure 1. The role of CETP in plasma cholesterol transport. Cells in extra-hepatic tissues eliminate any cholesterol that is surplus to their needs by transferring it as free (unesterified) cholesterol (FC) to HDLs in the extracellular space. The FC in HDLs is then either delivered to the liver in a process dependent on hepatic scavenger receptor (SR)-B1 or converted into cholesteryl esters (CE) by lecithin cholesterol acyltransferase (LCAT). The CE formed in HDLs is subsequently transported to the liver by either of two pathways: a direct pathway mediated by SR-B1 and an indirect pathway in which HDL CE is first transferred to the VLDL/LDL fraction by CETP and then taken up by the liver following binding of LDL to hepatic LDL receptors (LDL-R).

CETP inhibitors

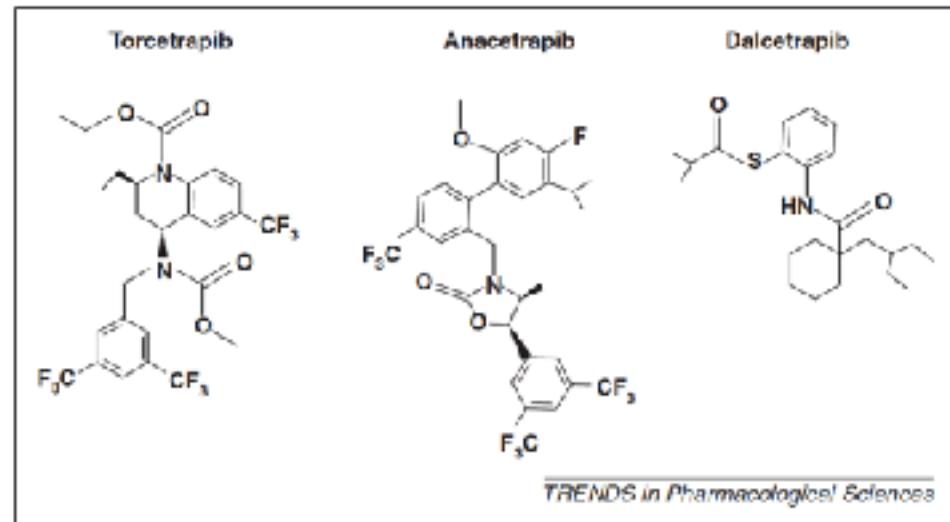


Figure 2. Structures of torcetrapib, anacetrapib and dalcetrapib.

- Torcetrapib - ILLUMINATE halted due to safety issues
- Dalcetrapib (raises HDL, does not lower LDL) stopped by Roche (no clinical benefit)
- Anacetrapib (raises HDL, lowers LDL) - DEFINE
- Evacetrapib (raises HDL, lowers LDL)

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-Sendor, 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*

After 12 months, significant HDL increase (72%)
and LDL decrease (25%), but increased risk for
CVD (HR=1.3) and death (HR=1.6)

Health

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LOLA
VERSUS
WATSON TOOK IT

Pfizer Ends Studies on Drug for Heart Disease

*By ALICE BORCHARD
Political staff, Government 2d, 1928*

Pfizer announced last night that it had discontinued research on its most important experimental drug, a treatment for heart disease. The decision is a sombering development that is likely to seriously damage the company's prospects through the next decades. Preliminary research found that the drug linked with deaths and injuries.

Preliminary research found that the drug, torcetrapib, appeared to be linked with deaths and heart problems in the patients who were taking it. For people with heart disease, Pfizer's decision to stop the trial represents the failure of a drug that many cardiologists had viewed as a potentially major advance in efforts to reduce heart attacks and strokes.

Torcetrapib is designed to raise levels of so-called good cholesterol in combination with older drugs called statins. Like statins,

Turcetrapib is designed to raise levels of so-called good cholesterol. It was to be used in combination with older drugs called statins, like Lipitor and Zocor, which reduce so-called bad cholesterol.

As recently as Thursday, Pfizer executives had huddled the drug at a meeting with investors and analysts at the company's research center in Groton, Conn.

This will be one of the most important compounds of our era, said Peter S. Saltonstall, Pfizer's chief executive.

It is the world's biggest pharmaceutical company.

As recently as Thursday, Pfizer executives had hailed the drug at a meeting with investors and analysts at the company's research center in Groton, Conn. "This will be one of the most important compounds of our generation," said Jeffrey B. Kindler, Pfizer's chief executive.

In a news release issued yesterday, the company said that it would immediately halt clinical trials of the drug and end its development. The decision was based on interim results from a 15-month study called Illuminata, which was designed to determine whether the combination of the two drugs could reduce the risk of heart attack and stroke. The results showed that the combination was effective, but also raised concerns about safety.

The decision was based on interim results from a 15,000-patient clinical trial. The trial, called Illustrate, was scheduled to be completed in 2009. Pfizer had hoped it would prove that the combination of the two drugs was significantly more likely to reduce heart attack and strokes than Lipitor alone.

Even before yesterday's announcement, some cardiologists had raised concerns about Lipitor alone dose.

The image shows the front cover of a book titled "THE VIRUS THAT CAUSES SHINGLES" by Dr. Jennifer Price. The cover features a dark background with a network of glowing red and orange lines resembling neurons or blood vessels. The title is prominently displayed in large, white, serif capital letters. Below the main title, the subtitle "MAY ALREADY BE INSIDE YOU" is written in a smaller, white, sans-serif font. At the bottom, the text "GET THE INSIDE STORY" is followed by a small circular logo containing a white 'i'. The overall design is professional and informative.

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 3. **Well: New Device Harnesses Productive Cancer Screening**
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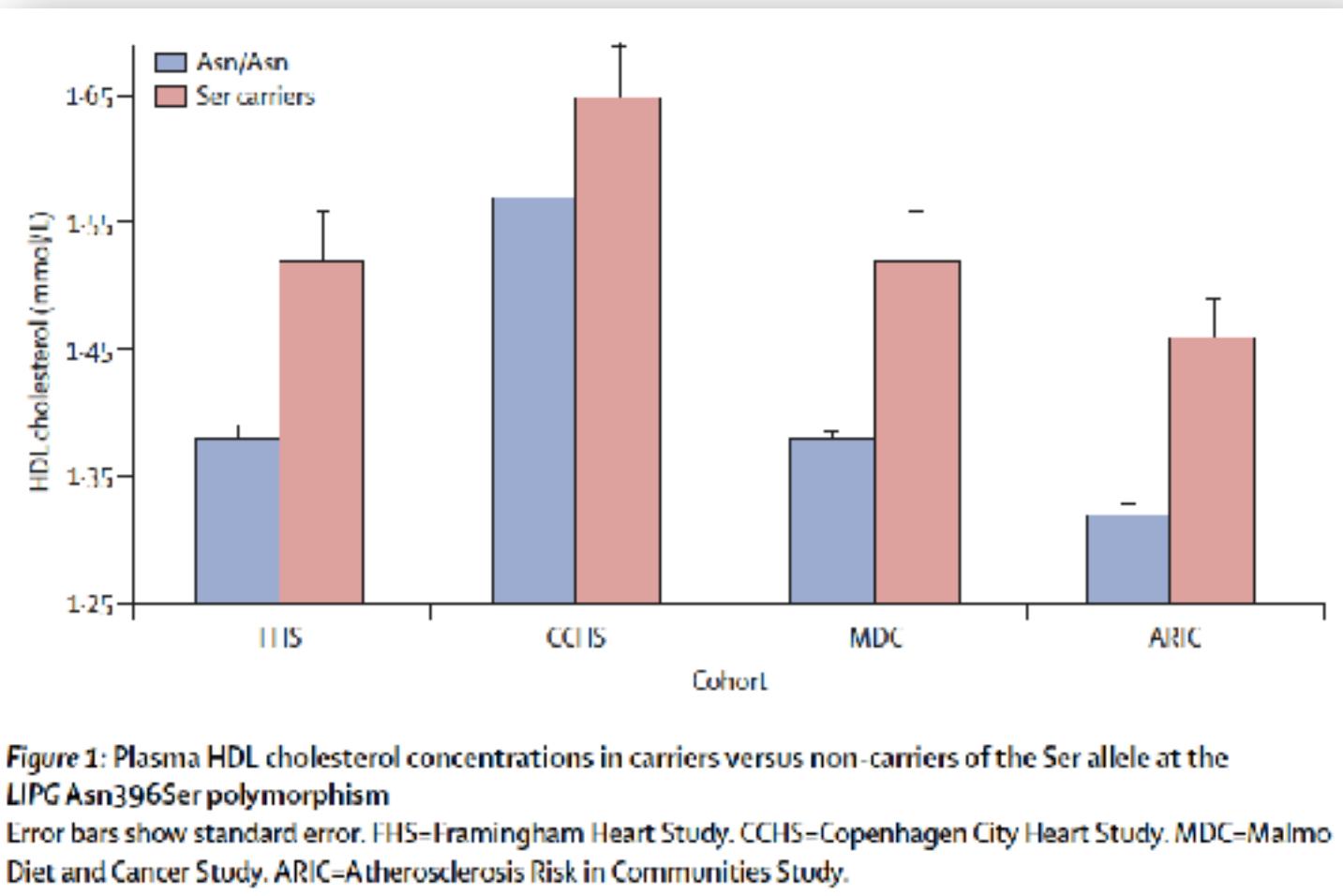
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Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study

Benjamin F Voight^a, Gina M Palosa^a, Marju Orho-Melander, Ruth Frikkie Schmidt, Maja Barbalic, Majken K Jensen, George H Lindy, Hilma H ö lm, Eric J Ding, Toby Johnson, Herbert Schunkert, Niles J Samani, Robert Clarke, Jemma C Hopewell, John I Thompson, Mingyan Li, Gudmar Thorleifsson, Christopher Newton Cheah, Kiran Musunuru, James P Pirruccello, Danish Saleheen, JJ Chen, Alexandre F R Stewart, Anne Schiller, Unnur Thorsdottir, Gudmundur Thorsteirsson, Sonja Anund, James C Engert, Thomas Morgan, John Sperling, Monika Stoff, Klaus Berger, Nicola Martinelli, Domenico Gianni, Pascal P McKeown, Christopher C Patterson, Stephen E Epstein, Joseph Devaney, Mary Susan Burnett, Vincent Mooser, Samuli Ripatti, Ida Surakka, Markku S Nieminen, Juha Siitonen, Marja Liisa I okki, Markus Perola, Aki I Jauhainen, Ulf de laat, Bruno Gigante, L n Ingesson, Tanja Zeller, Philipp Wild, Paul J W de Bakker, Olaf J K lungel, Anke-Hilse Maitland-van der Zee, Bas J M Peters, Antonius de Boer, Diederick E Grubbe, Pieter W Kammhuisen, Vera H M Denier, Clara C Elbers, N Charlotte Onland Moret, Marten H Hofker, Cisco Wijmenga, WM Monique Verschuren, Jolanda M A Boer, Yvonne T van der Schouw, Asif Rasheed, Philippe Grossard, Serkalem Demissie, Cristen Willer, Ron Da, Jose M Ordovas, Gonçalo R Abecasis, Michael Boehnke, Karen I Mohlke, Mark J Daly, Constance Guiducci, Noël P Burtt, Auri Surti, Llana Gonzalez, Shuang Purcell, Stacey Gabriel, Jaume Murrugarra, John Peden, Jeannette Liedmann, Patrick Diemert, Christina Willenborg, Inke R Konig, Marcus Fischer, Christian Hengstenberg, Andreus Ziegler, Jan Buyschoot, Diether Lambrechts, Frans Van de Werf, Keith A Fox, Nour Eddine El Mukhtari, Diana Rubin, Jürgen Schrezenmeir, Stefan Schreiber, Arne Schäfer, John Danesh, Stefan Blankenberg, Robert Roberts, Ruth McPherson, Hugh Watkins, Alistair S Hall, Kim Overvad, Eric Rimm, Eric Boerwinkle, Anne Tybjaerg Hansen, I Adrienne Cupples, Muredach P Reilly, Olli Melander, Pier M Mannucci, Diego Ardissino, David Siscovick, Roberto Giosu, Karl Stefansson, Christopher J O'Donnell, Veikko Salomaa, Daniel J Rader, Leena Peltonen, Stephen M Schwartz, David Altshuler, Sekar Kathiresan

Asn396Ser in *LIPG* increases HDL-C, and does not affect other relevant factors
(BP, T2D, BMI, CRP, LDL)

HDL increased in Ser carriers



But no protection against MI

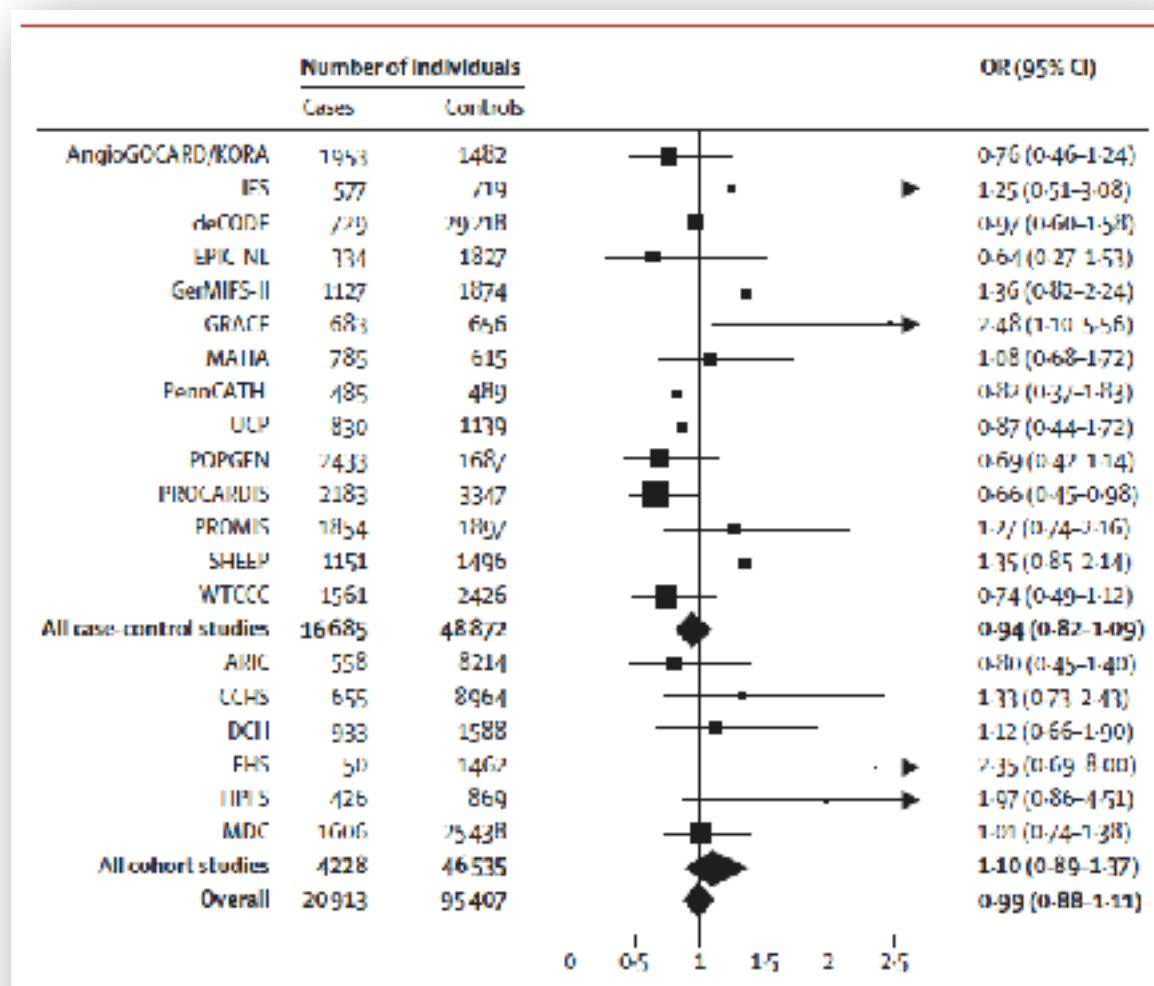


Figure 2: Association of LIPG Asn396Ser with myocardial infarction in 116 320 participants from 20 studies
In each study, the HDL cholesterol raising serine allele was modelled.

HDL has no impact on myocardial infarction risk

Odds ratio (95% CI) per SD increase in plasma lipid based on observational epidemiology*	Odds ratio (95% CI) per SD increase in plasma lipid conferred by genetic score†
LDL cholesterol	1·54 (1·45–1·63) 2·13 (1·69–2·69), $p=2\times 10^{-10}$
HDL cholesterol	0·62 (0·58–0·66) 0·93 (0·68–1·26), $p=0·63$

*Observational epidemiology estimates derived from more than 25 000 individuals from prospective cohort studies as shown in the appendix p 22. †LDL genetic score consisting of 13 single nucleotide polymorphisms (SNPs) as shown in the appendix p 27; HDL genetic score consisting of 14 SNPs as shown in the appendix p 28.

Table 4: Estimate of the association of genetically raised LDL cholesterol or HDL cholesterol and risk of myocardial infarction using multiple genetic variants as instruments

Original Investigation

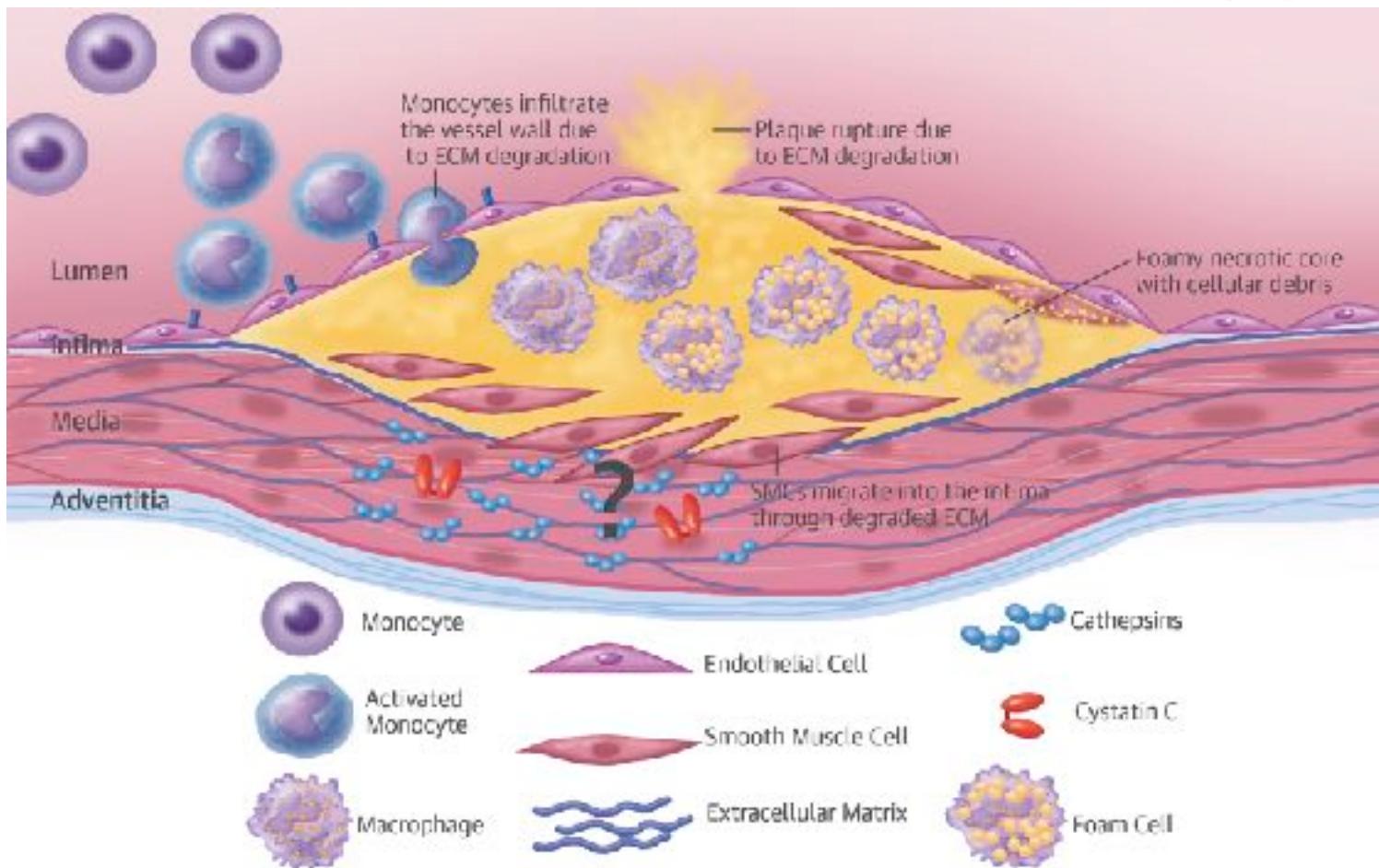
Cystatin C and Cardiovascular Disease: A Mendelian Randomization Study

Rosalie M. van der Laan, PhD,^{1,2} Arjan J. M. Blaauw, PhD,^{1,2} Wim J. van der Harst, PhD,^{1,2} Michaela Tzoulaki, PhD,³ A. Smita Sedaghati, PhD,⁴ Jeroen Buitens, PhD,⁵ Delphine Zelissen, PhD,⁶ Jessica van Seijen, PhD,^{7,8} Ingrid Jager, PhD,⁹ Susanna P. Cederlund, PhD,¹⁰ Adriaan Dierckx, MD,¹¹ Philippa Darroch, PhD,¹² Nicola Trampush, PhD,¹³ Melanie Wildenauer, PhD, MPH,¹⁴ Barbara Doris, MD,¹⁵ Paul K. Magnusson, PhD,¹⁶ Vincenzo De Cesare, PhD,¹⁷ Andrea Corrao, MD, PhD,¹⁸ Fabio R. Assedanoglu, MD,¹⁹ and 20 others

See page 954

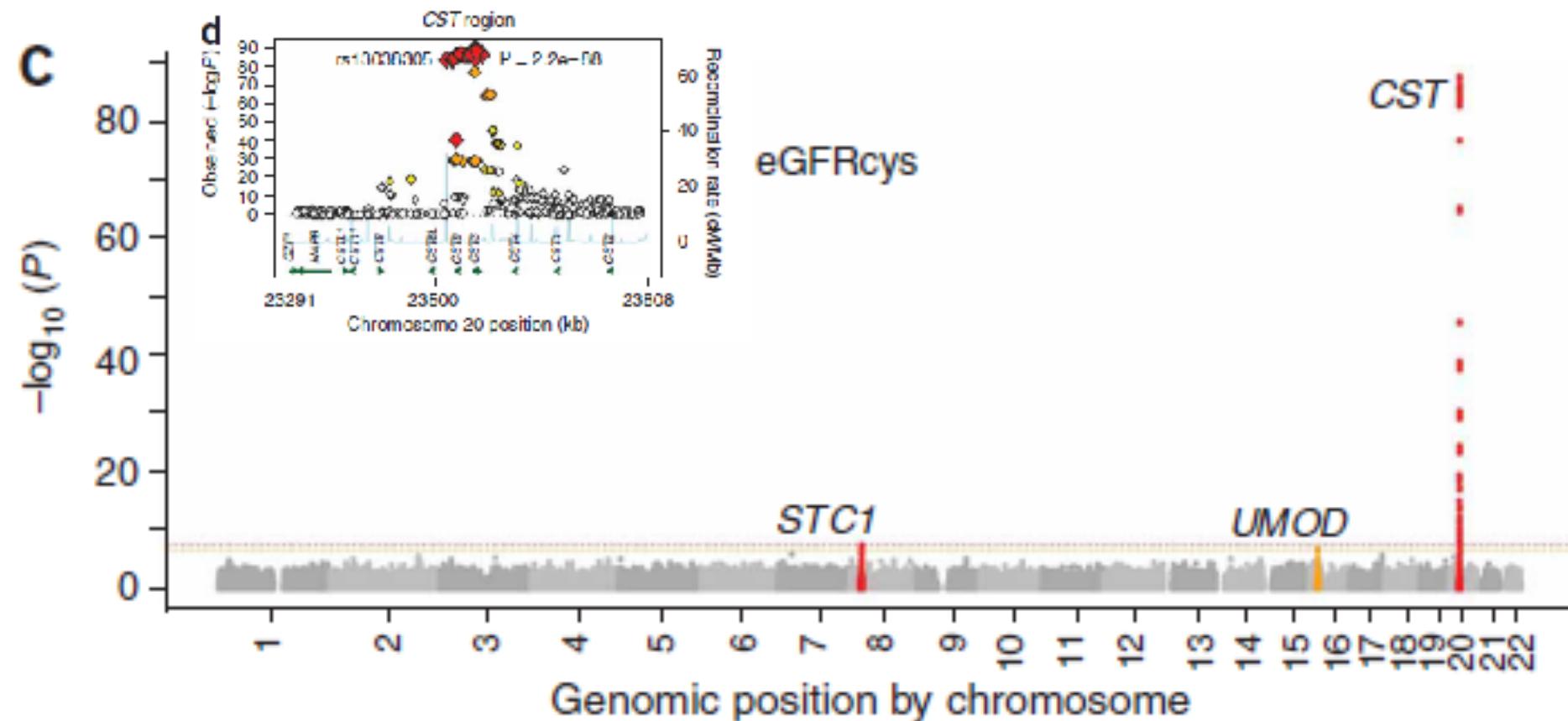
<https://doi.org/10.1016/j.jacc.2019.06.062>

Getting to the point



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

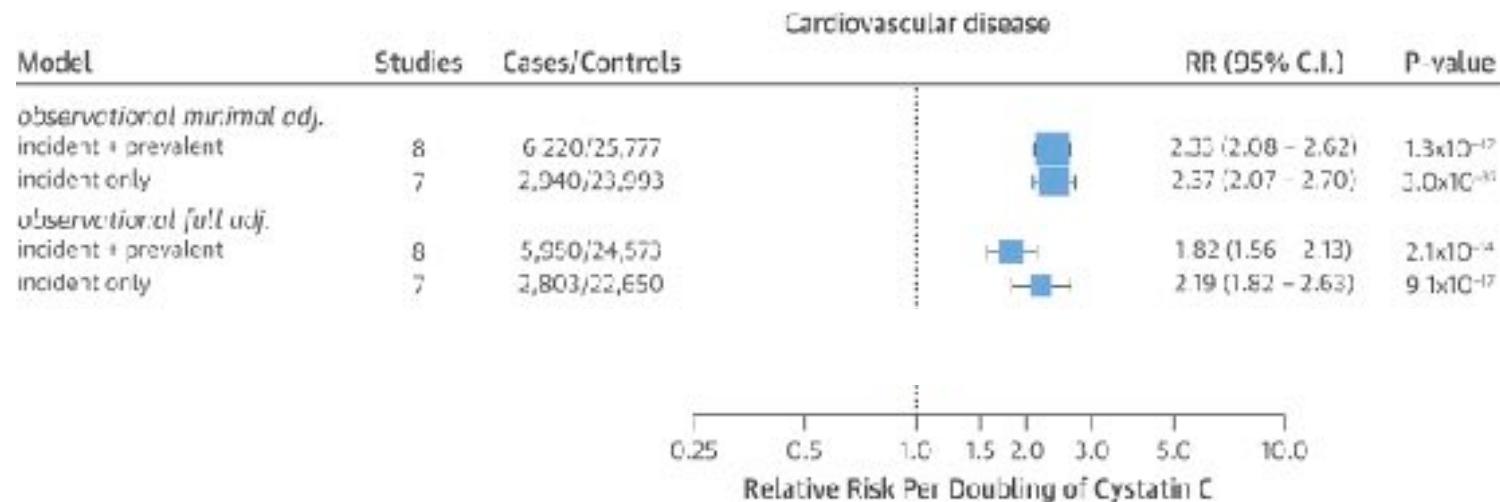
Study	Total	SNP*	Cystatin C†	CVD‡	CHD‡	IS‡	HF‡	MI‡	Male	Age (yrs)	Cystatin C (mg/dl)
3C	6,440	6,435	1,244	1,717	1,235	459	439	486	39.19	74.30 ± 5.52	0.92 ± 0.24
EPIC-NL	6,265	5,192	—	1,967	1,430	537	—	1,430	22.39	53.80 ± 10.23	—
GOSH	1,478	1,479	—	493	111	235	233	—	42.08	51.08 ± 11.86	—
HRS	7,844	5,585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

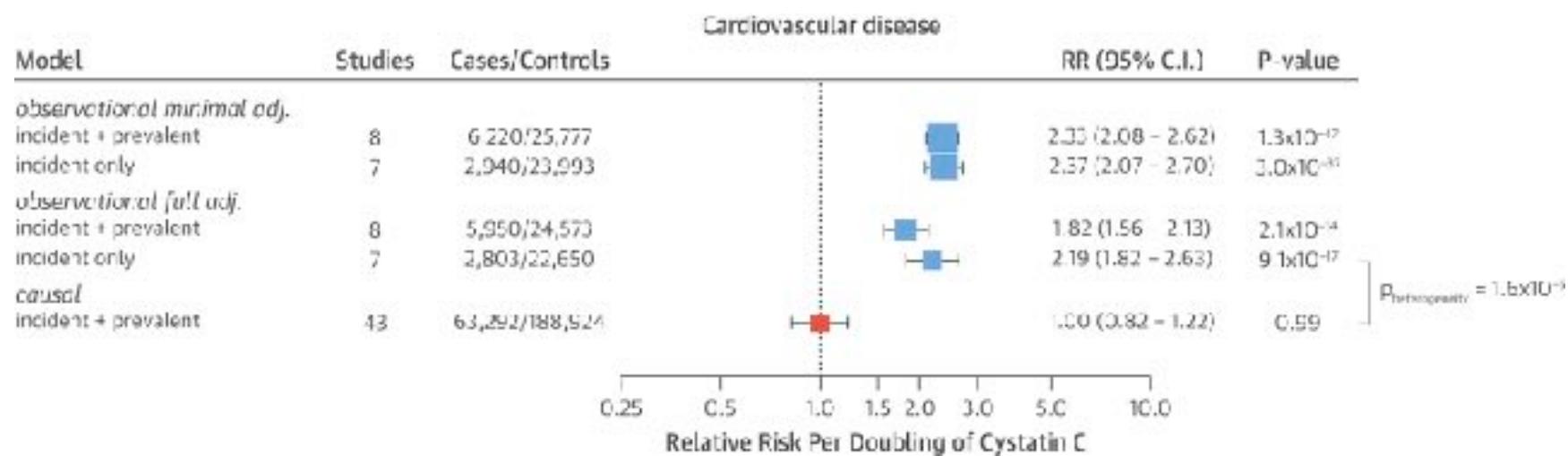
‡Indicates total incident and prevalent cases of disease or composite diseases in the case of CVD. §PROSPER is a randomized clinical trial. ||For the association of SNP with cystatin C concentrations, 9,488 samples were available in TWINGENE.

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

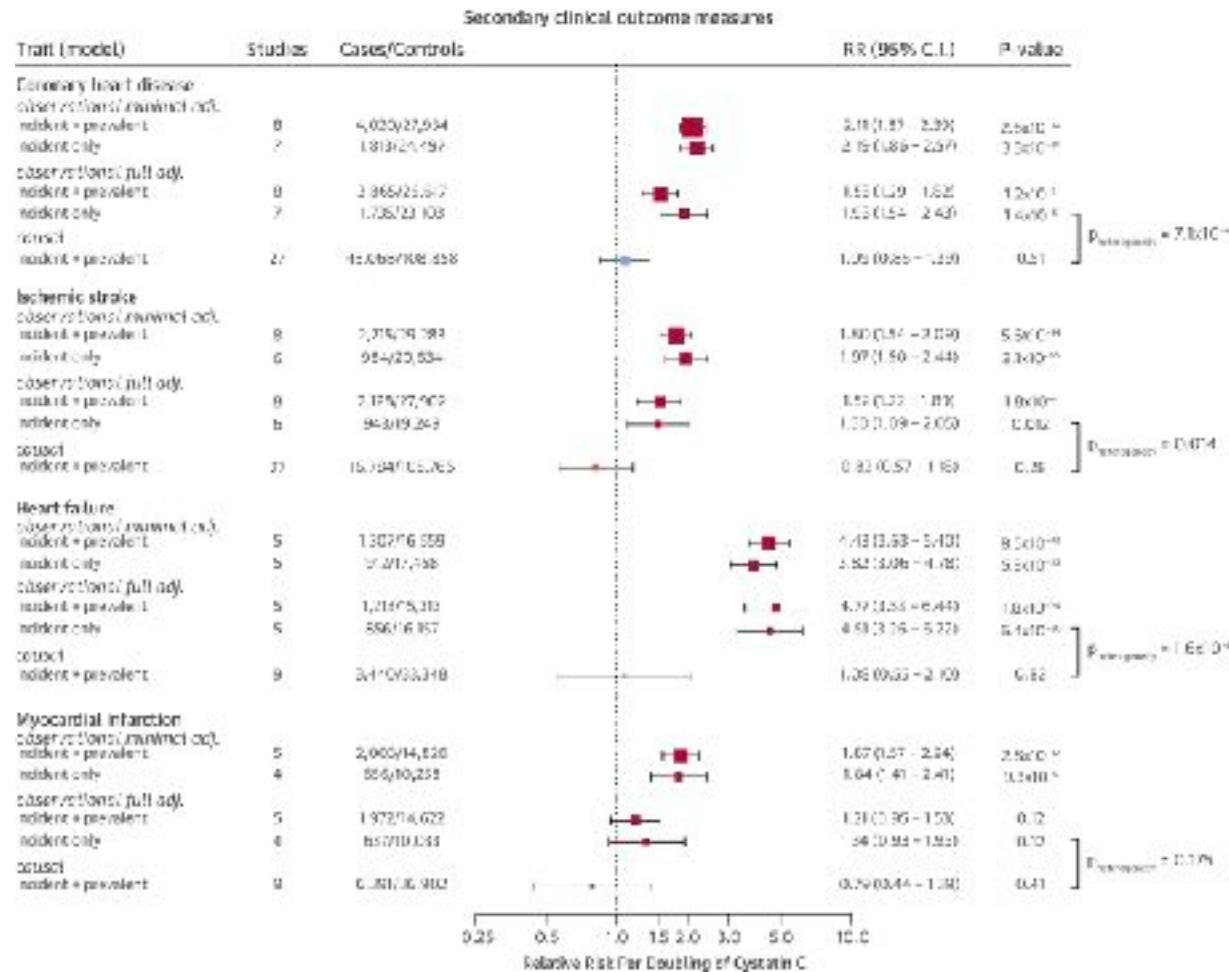
Cystatin C associates to CVD in observational studies



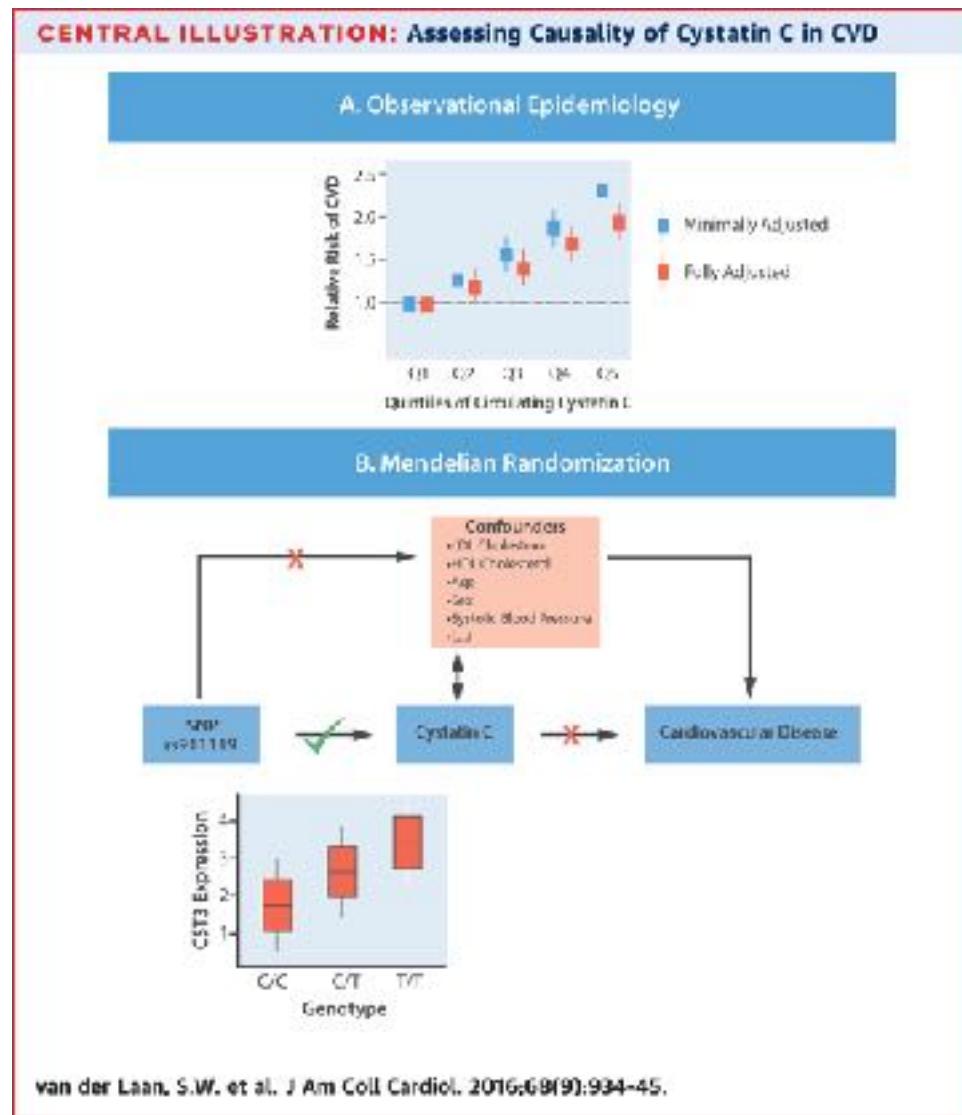
No causal effect of Cystatin C on CVD



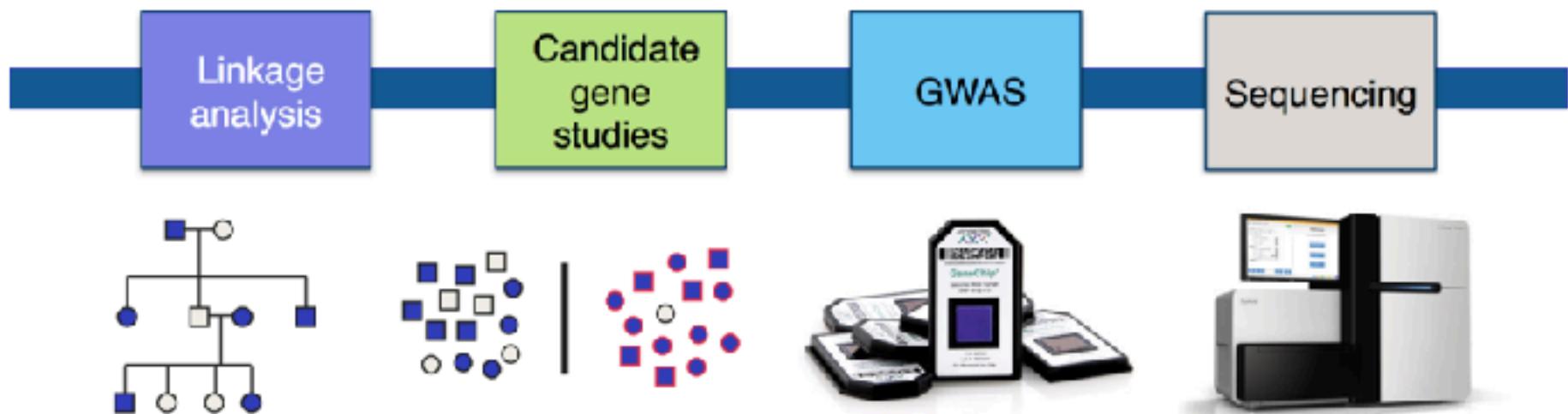
Secondary clinical endpoints: nada, nothing, zip



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 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

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Donald W. Reynolds Foundation
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LDL and PCSK9 in two populations

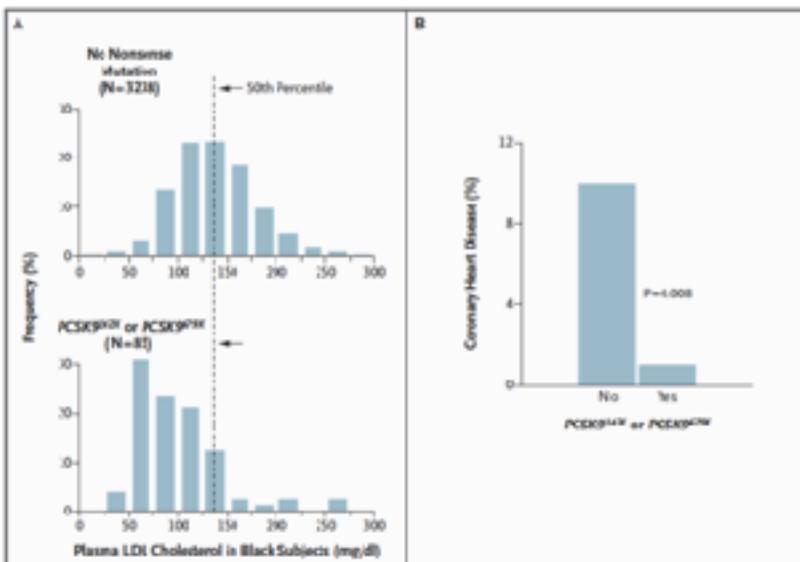


Figure 1. Distribution of Plasma LDL Cholesterol Levels (Panel A) and incidence of Coronary Heart Disease (Panel B) among Black Subjects, According to the Presence or Absence of a *PCSK9^{107R}* or *PCSK9^{108W}* Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 3238 black subjects who did not have a *PCSK9^{107R}* or *PCSK9^{108W}* allele (top) is compared with the distribution of levels among the 81 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 0.02584.

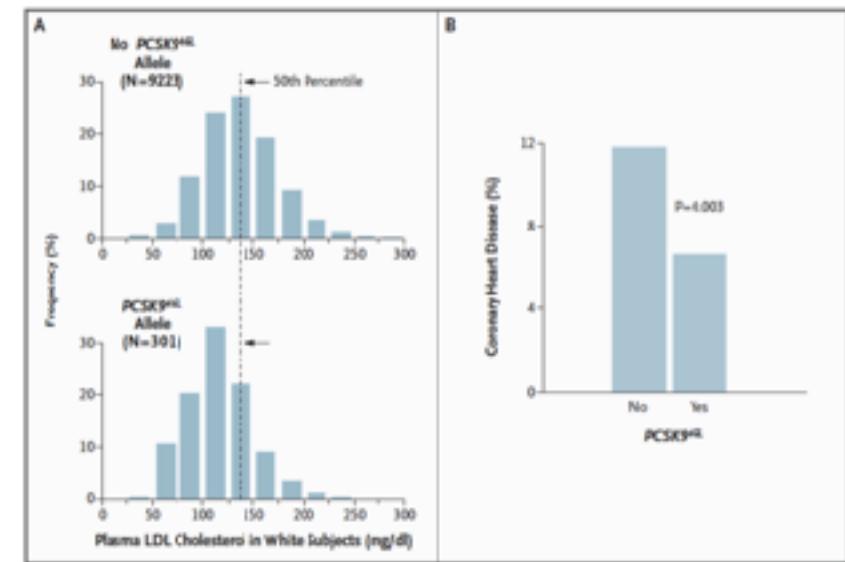
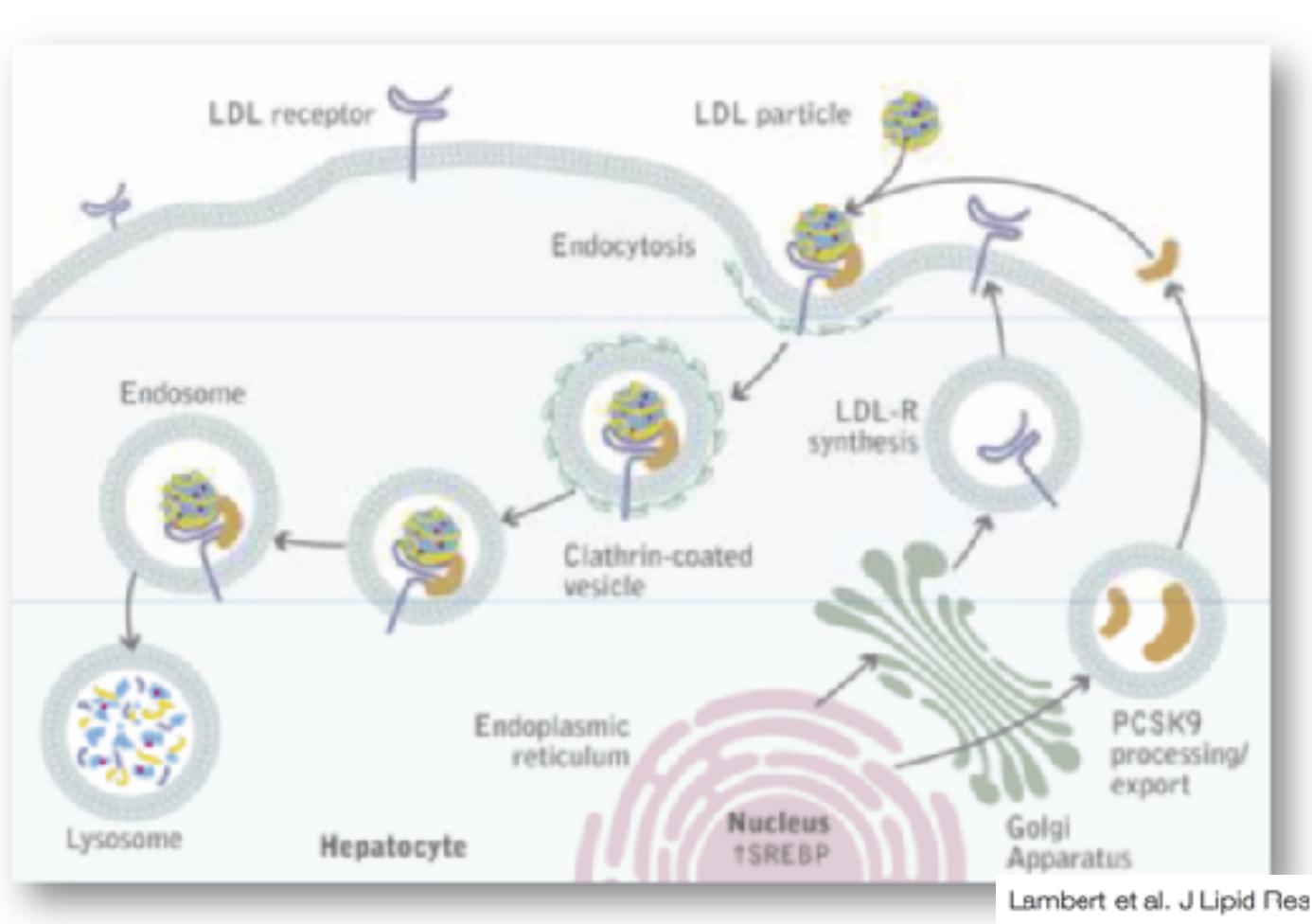


Figure 2. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Events (Panel B) among White Subjects, According to the Presence or Absence of a *PCSK9^{107R}* Allele.

In Panel A, the distribution of plasma LDL cholesterol levels at baseline among 9223 white subjects who did not have a *PCSK9^{107R}* 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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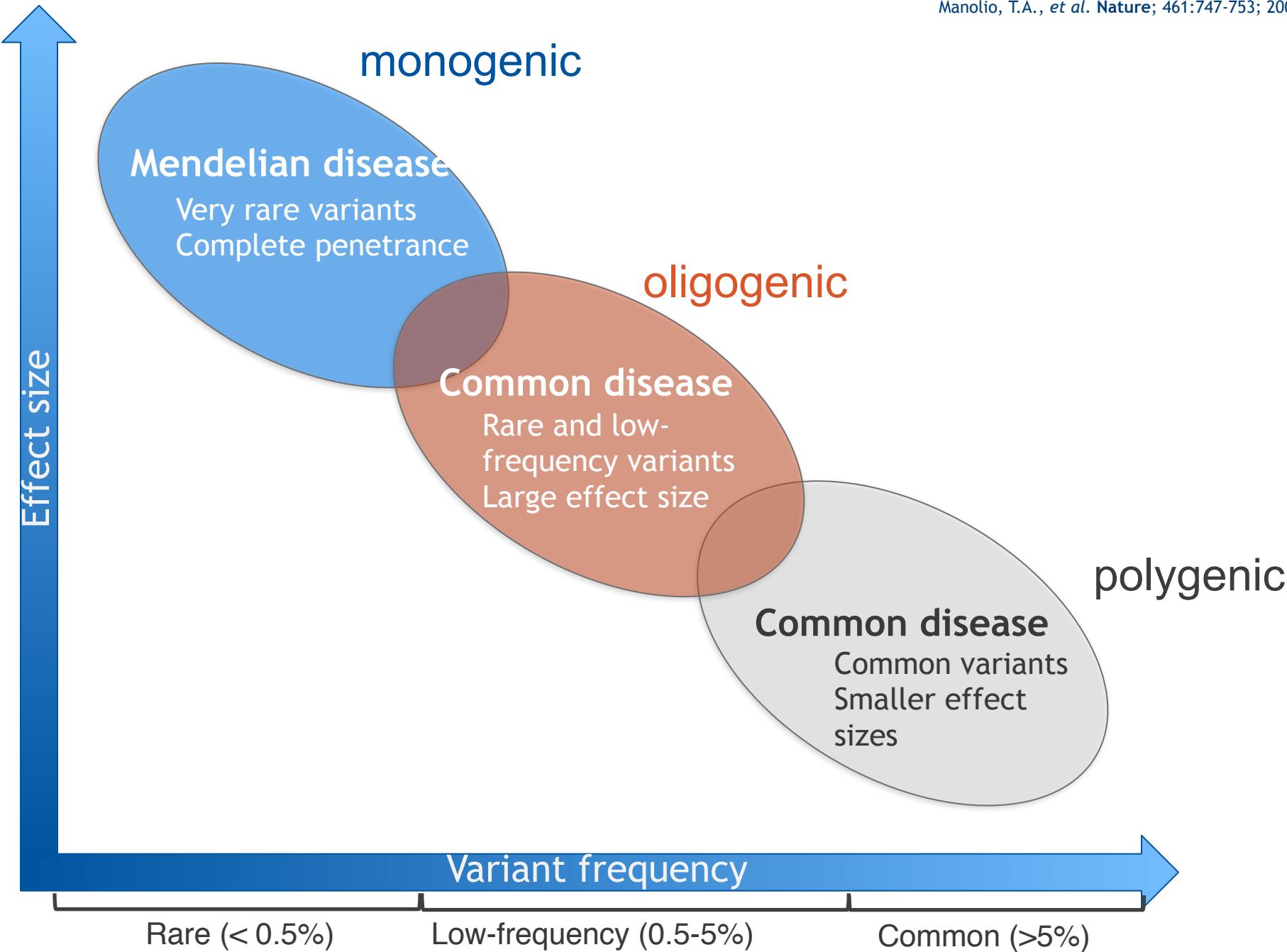
Athero-Express | AAA-Express | CTMM | many

more

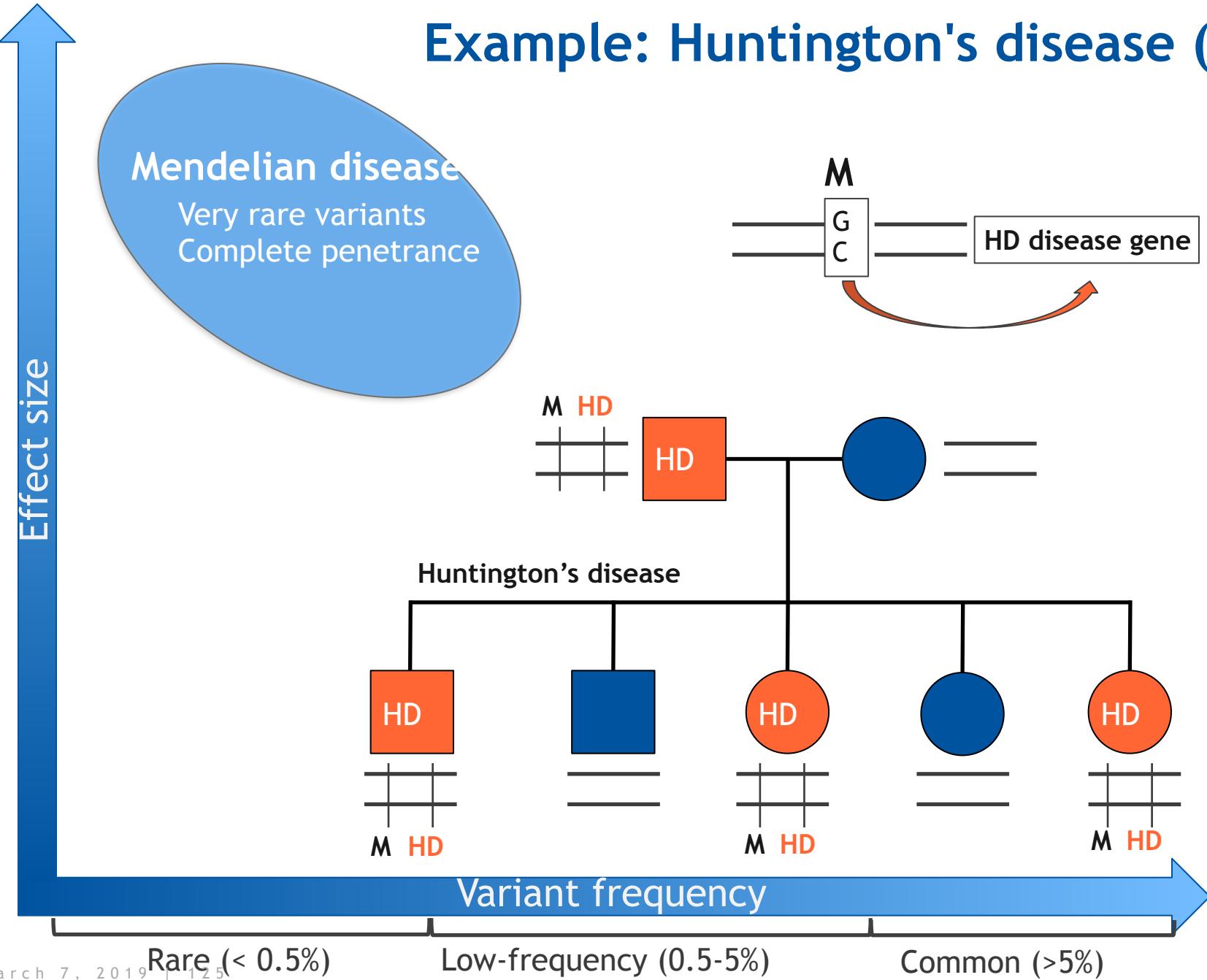


Cardiovascular Genomics





Example: Huntington's disease (HD)



A polymorphic DNA marker genetically linked to Huntington's disease

James F. Gusella^{*}, Nancy S. Wexler[†], P. Michael Conneally[‡], Susan L. Naylor[§],
Mary Anne Anderson[§], Rudolph E. Tanzi[§], Paul C. Watkins^{||}, Kathleen Ottina[§],
Margaret R. Wallace[‡], Alan Y. Sakaguchi[§], Anne B. Young[†], Ira Shoulson[†],
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† Hereditary Disease Foundation, 9701 Wilshire Blvd, Beverly Hills, California 90212, USA

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|| Venezuela Collaborative Huntington's Disease Project

Family studies show that the Huntington's disease gene is linked to a polymorphic DNA marker that maps to human chromosome 4. The chromosomal localization of the Huntington's disease gene is the first step in using recombinant DNA technology to identify the primary genetic defect in this disorder.

1983

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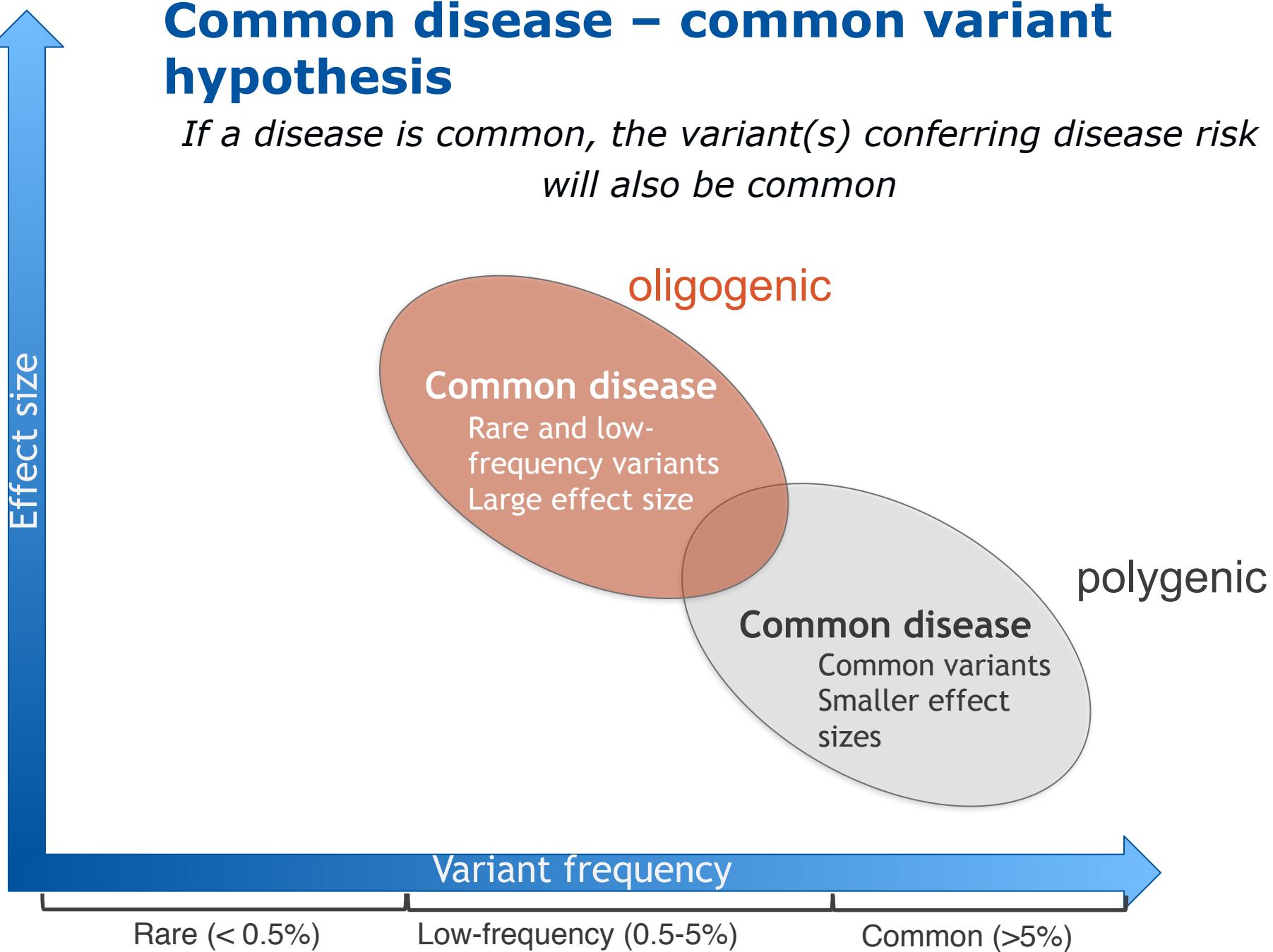
1983



1989

Common disease – common variant hypothesis

If a disease is common, the variant(s) conferring disease risk will also be common



What type of disease are we looking at?

• Complex diseases

- Polygenic, multifactorial diseases
 - Diabetes mellitus
 - Asthma
 - Cardiovascular disease
 - Hypertension

Each gene contributes a little to the disease

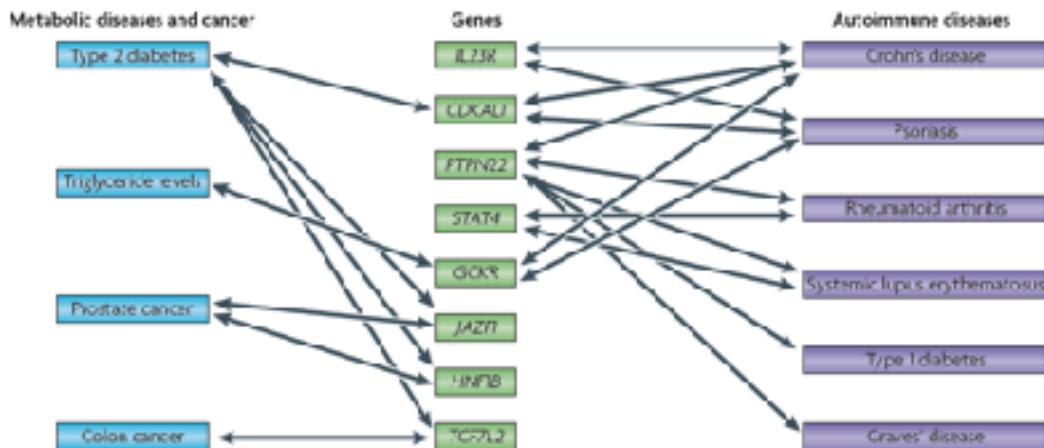
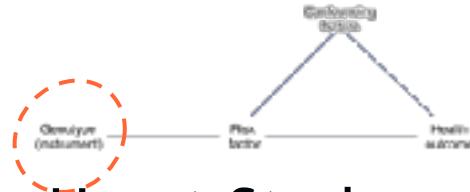


Table 3 Some Recent Genes/Loci Identified in Coronary Artery Disease

Gene/Locus	Functional Genomics	Independently Replicated	Reference(s)
CFH	Inflammation	Yes	49,50
LTA4H	Inflammation	No	12
FLAP	Inflammation	No	5
Lymphotactin	Inflammation	No	69
Collectin 2	Inflammation	No	68
Stromelysin 1	Inflammation	No	81
MHC2TA	Inflammation	No	71
Kinin	Inflammation	Yes	13
TSP-4	Endothelial integrity	Yes	57-61
Connexin 37	Endothelial integrity	No	81
MER2A	Endothelial integrity	Yes	63,63
Apo E4	Lipoprotein handling	Yes	50,53,78
LRP6	Lipoprotein handling	No	56
PCSK9	Lipoprotein handling	No	42,43
VAMP8	Thrombosis	No	72
PAI-1	Thrombosis	No	81
Factor V (169da)	Thrombosis	No	73
Prothrombin (20210W)	Thrombosis	No	73
Ap21	Unknown	Yes	38-40

Abbreviations: Apo E4 = apolipoprotein E4; FLAP = 5-lipoxygenase activating protein; LRP6 = low-density lipoprotein receptor-related protein 6; LTA4H = leukotriene A4 hydrolase; MHC2TA = major histocompatibility factor class 2 transactivator; PAI-1 = plasminogen activator inhibitor 1; PCSK9 = proprotein convertase subtilisin/kexin type 9; TSP-4 = thrombospondin 4; VAMP8 = vesicle-associated membrane protein 8; other abbreviations as in Table 2.



SNP selection

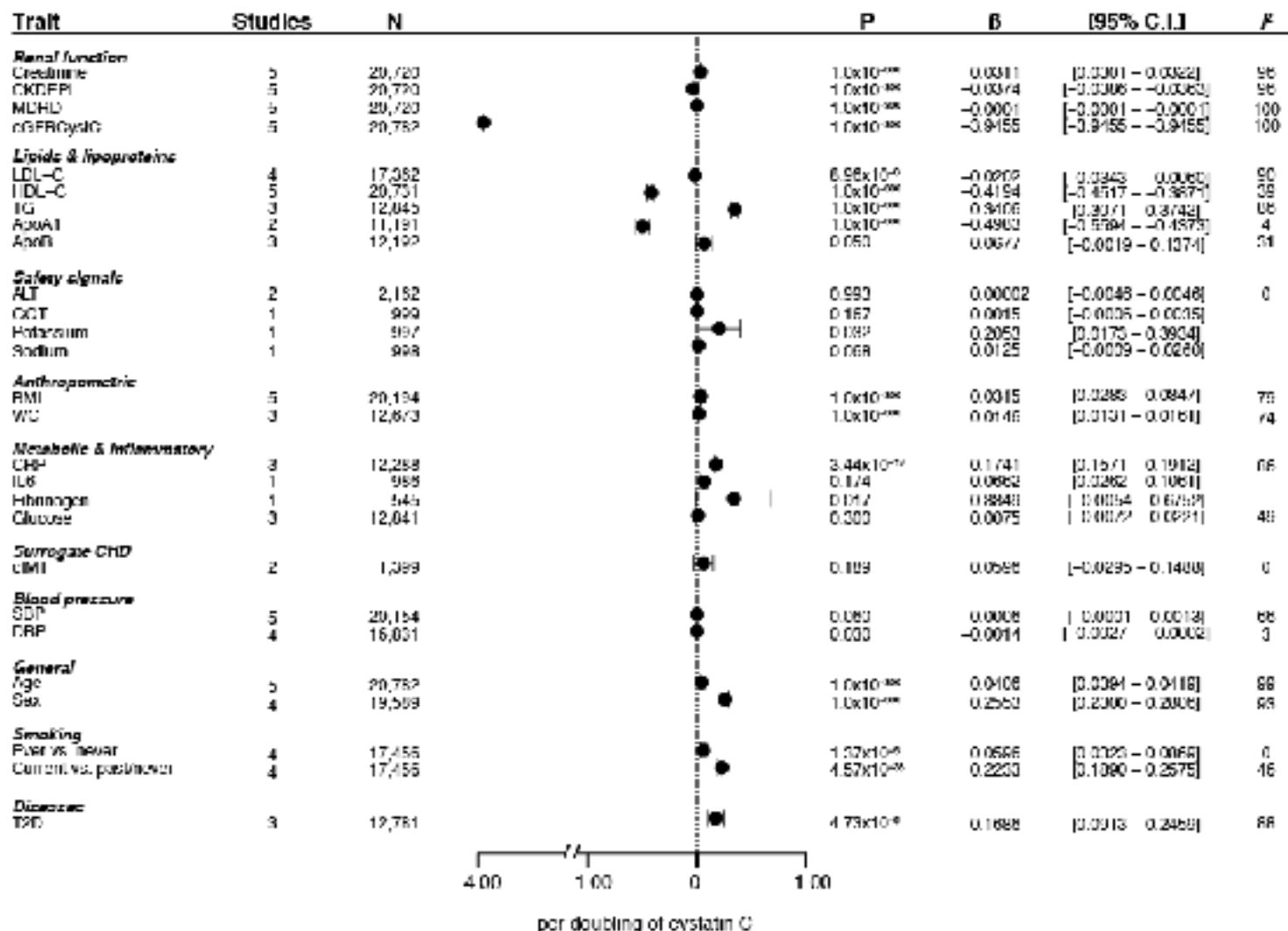
- One GWAS on CystC, rs1158167 in Framingham Heart Study
- Three (meta-analyses of) GWAS on eGFR_{CystC}
 - rs911119, rs13038305
 - eGFR_{CystC} = 76.7 x (serum CystC)^{-1.19}
 - Log linear relation between serum CystC and eGFR

Proxy	Distance	r^2	Chr	Position	Minor	Major	MAF	Variant	Gene
<u>rs1158167</u>	34,548	0.913	20	23,526,189	G	A	0.25	downstream	n/a
<u>rs17751897</u>	20,023	0.955	20	23,540,714	C	T	0.242	intergenic	n/a
<u>rs12625716</u>	5,892	0.955	20	23,554,845	A	G	0.242	downstream	n/a
<u>rs6048952</u>	5,480	0.955	20	23,555,257	G	A	0.242	downstream	n/a
<u>rs13038305</u>	2,475	1.00	20	23,558,262	T	C	0.233	intronic	CST3
<u>rs911119</u>	0	1	20	23,560,737	C	T	0.233	intronic	CST3

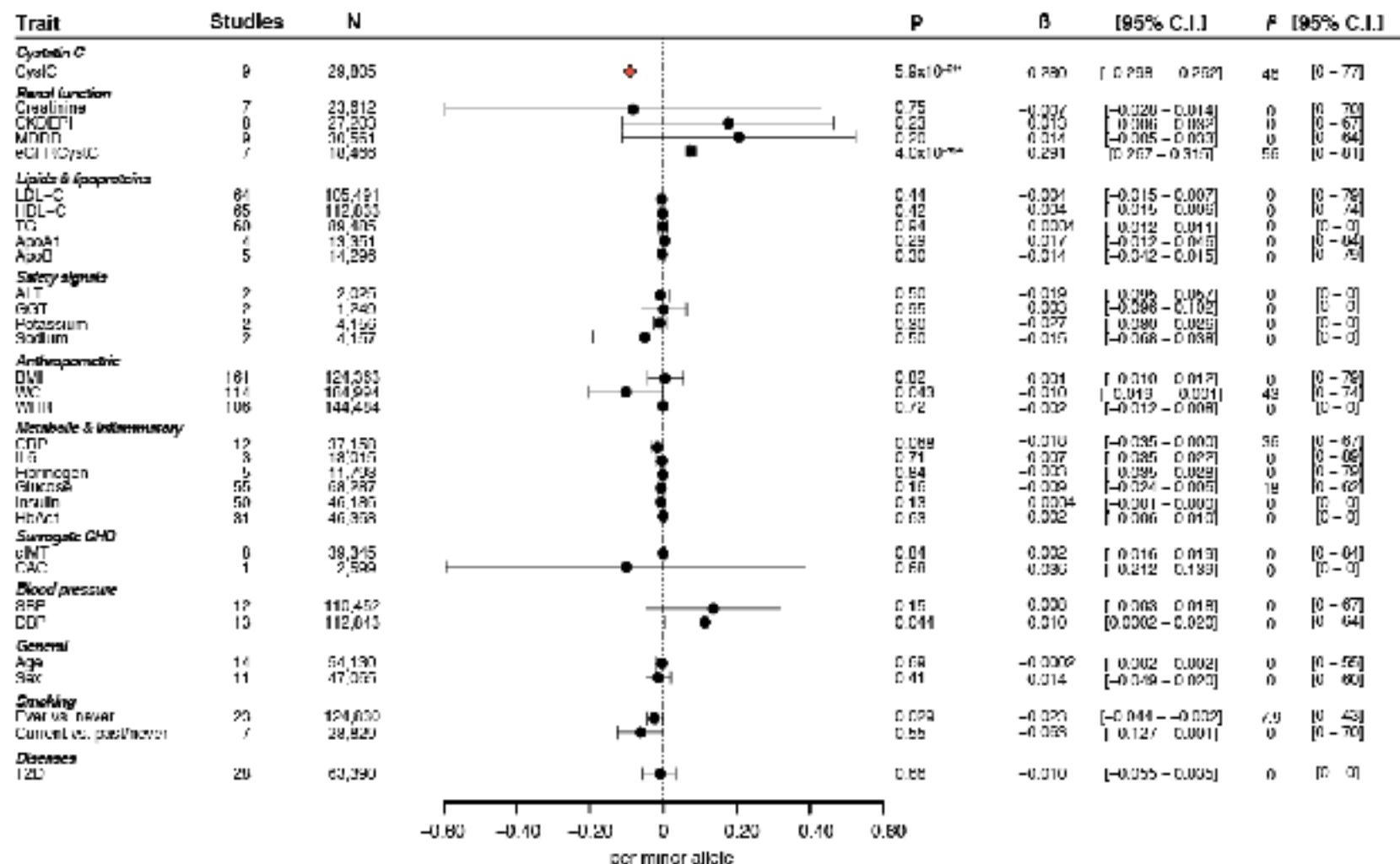
Studies included

Study	N _{total}	N _{SNP}	N _{CystC}	N _{CVD}	N _{CHD}	N _{stroke}	N _{HF}	N _{MI}
3C	6,440	6,435	1,244	1,717	1,235	459	439	486
EPIC-NL	6,265	5,192	-	1,967	1,222	443	-	1,430
GOSH	1,478	1,479	-	493	111	235	233	-
HRS	7,844	5,585	5,777	-	-	-	-	-
KORA	4,856	1,867	4,676	540	341	86	-	341
NBS	1,819	1,297	-	66	113	49	-	170
PIVUS	1,016	949	1,004	255	175	63	66	105
PREVEND	3,245	3,245	3,245	236	190	58	-	-
PROSPER	5,244	5,150	-	2,561	1,994	766	210	762
Rotterdam	4,730	5,974	3,906	3,579	1,934	1,328	1,625	1,176
SHIP	3,224	3,224	3,212	114	6	27	-	134
Tromsø	6,129	-	6,129	1,251	-	494	-	881
TWINGENE	6,902	6,902	6,740	940	610	287	206	-
ULSAM	1,221	1,107	1,193	503	285	159	199	-
WHI	7,854	7,844	-	4,831	2,930	2,111	-	2,934
Whitehall II	4,961	5,011	-	349	253	111	-	254
CARDIoGRAM				20,251	20,251			
C4D				15,388	15,388			
METASTROKE				12,389		12,389		
CHARGE-HF				2,526			2,526	
Overall	73,228	61,261	37,126	63,292	47,038	19,065	5,504	8,673

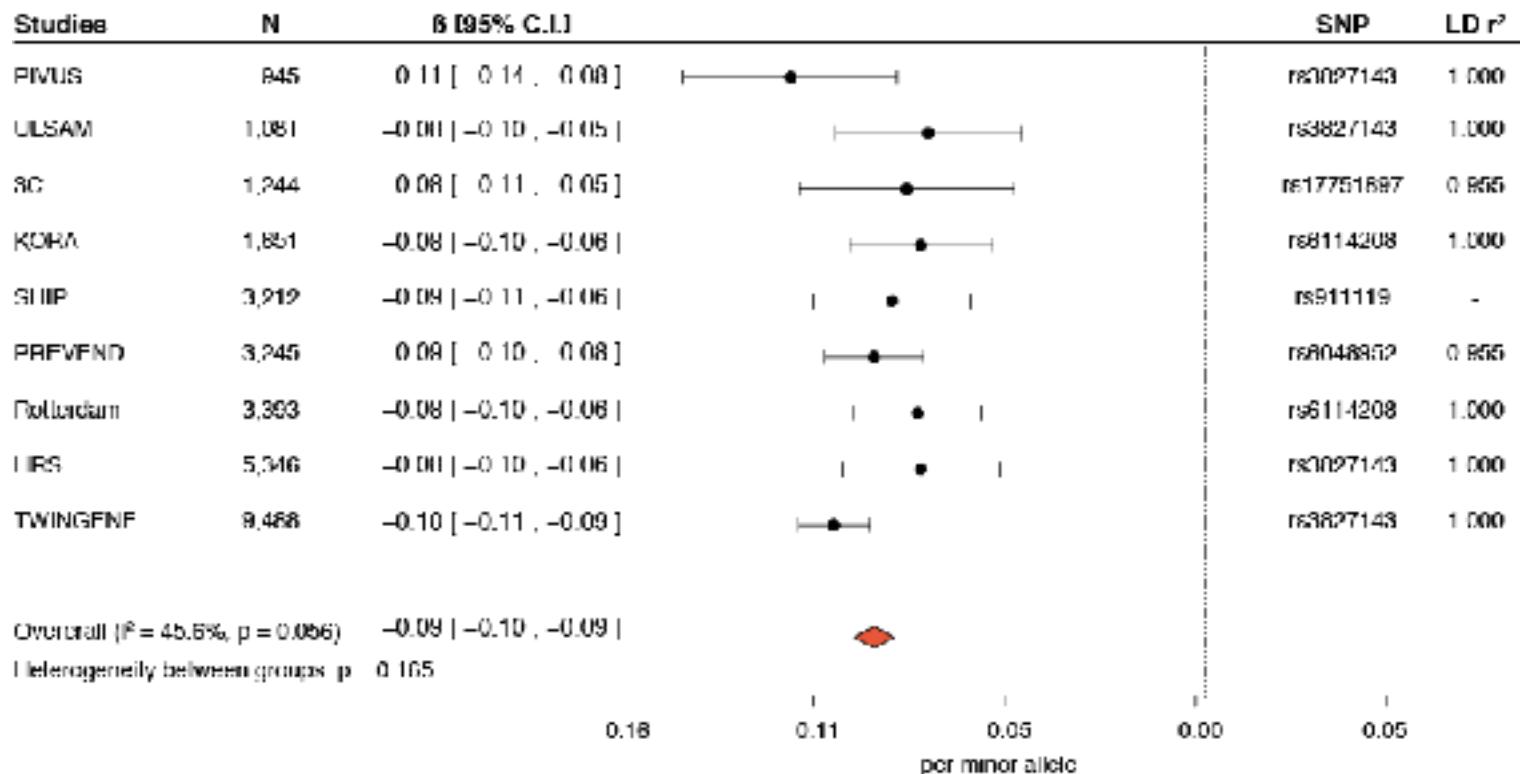
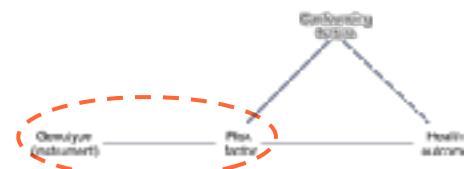
Serum cystatin C vs. risk factors



Cystatin C variant vs. risk factors

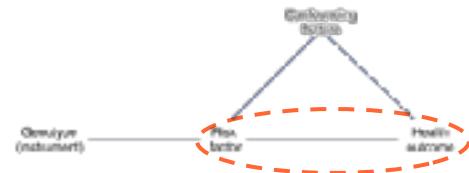


SNP vs. cystatin C

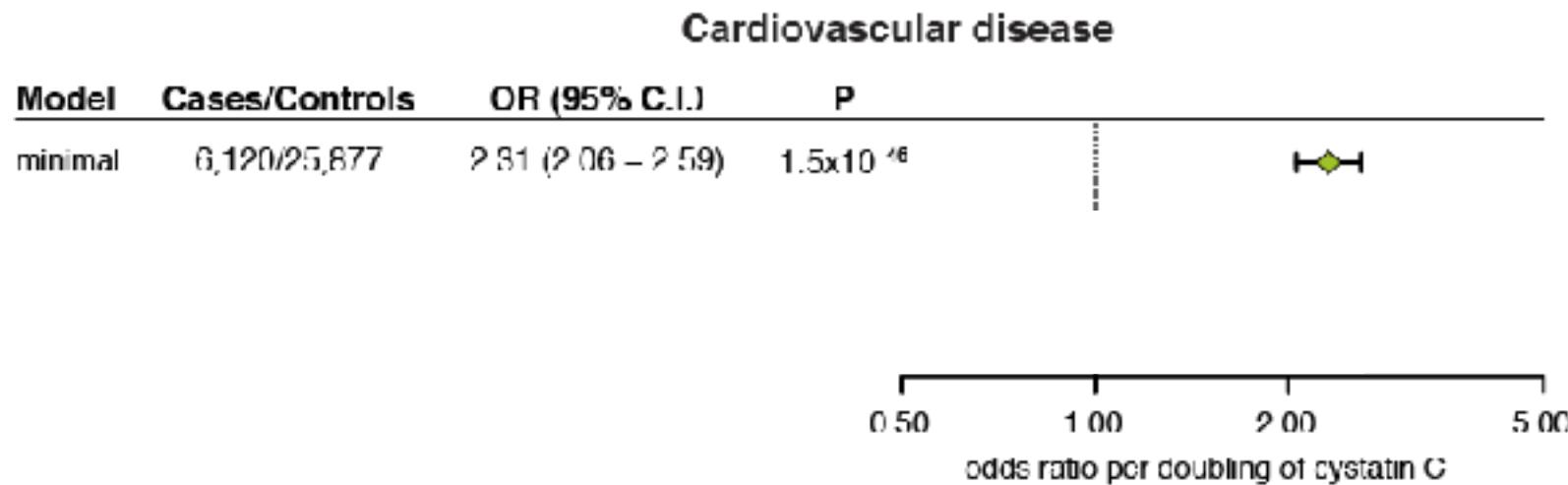


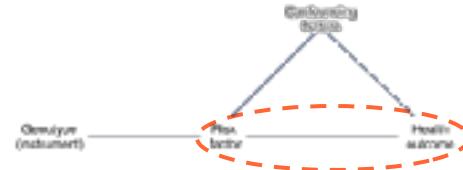
- Per minor allele there is 6.13% decrease in CystC [mg/L]
- $\beta = -0.09 [-0.10 - -0.09]$, $p = 5.95 \times 10^{-163}$, $N = 29,805$
- This explains $\approx 2.75\%$ of the phenotypic variation

Observational analysis



- Minimally adjusted model: age + sex

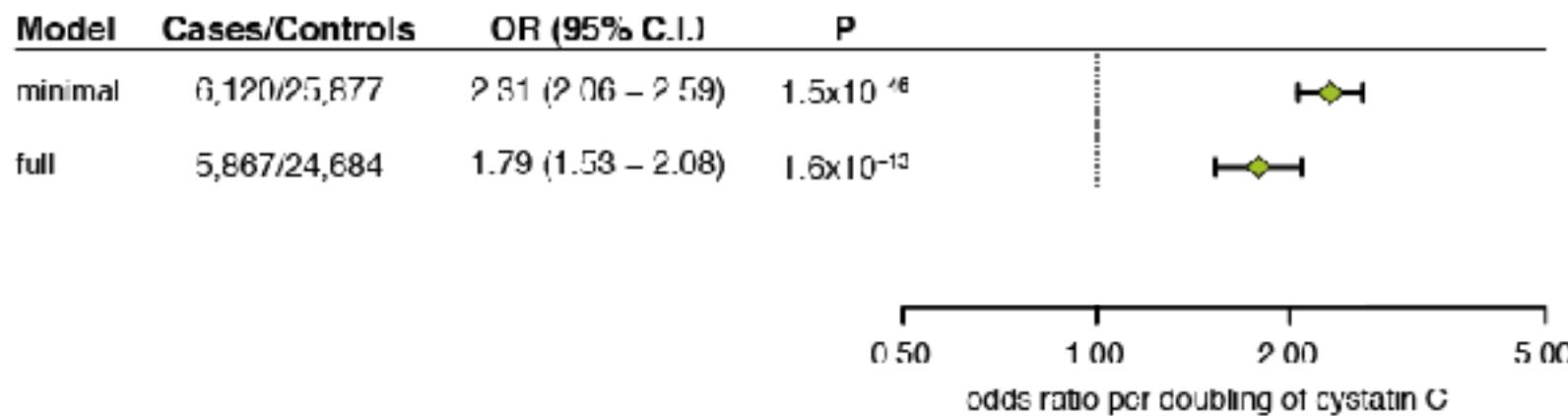




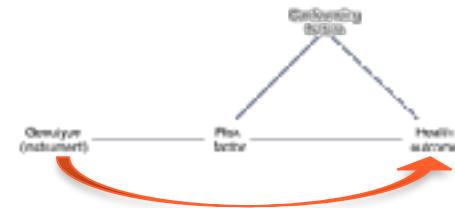
Observational analysis

- Minimally adjusted model: age + sex
- Fully adjusted model: age + sex + HDL + systolic blood pressure + BMI + smoking status + CKDEPI

Cardiovascular disease

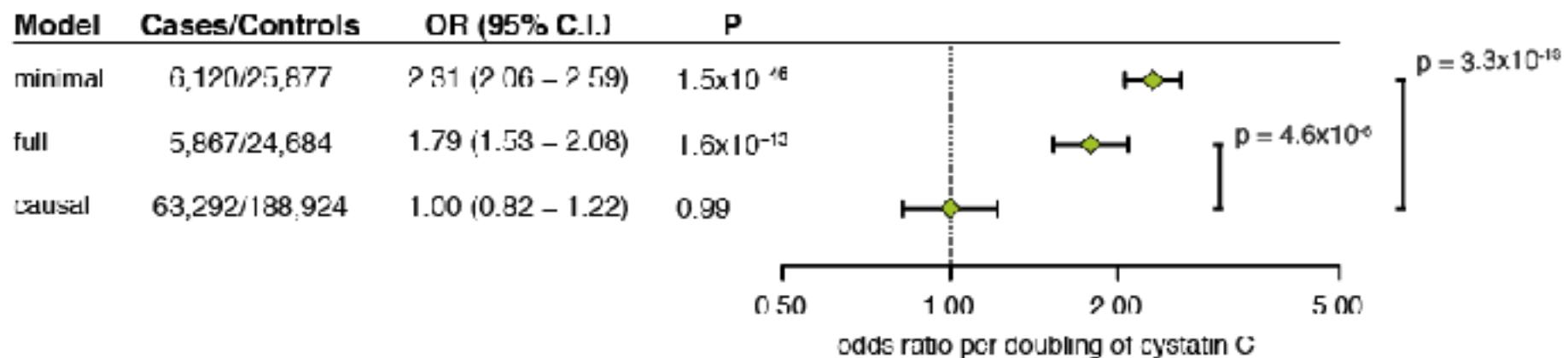


Mendelian randomization analysis

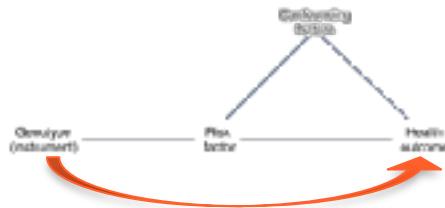


- Minimally adjusted model: age + sex
- Fully adjusted model: age + sex + HDL + systolic blood pressure + BMI + smoking status + CKDEPI
- Causal effect: *nada, nothing, niets, rien, nichts, ничего*
- *Interaction analysis:* significant - models are different

Cardiovascular disease



Other outcomes



- Causal effect: *nada, nothing, niets, rien, nichts, ничего*

