

GWAS and Mendelian Randomization

Applying modern-day genomic knowledge within
the atherosclerotic field

Sander W. van der Laan | s.w.vanderlaan-2@umcutrecht.nl | @swvanderlaan



UMC Utrecht



Disclosure: this work is partly financed by Cavadis

CAVADIS
www.cavadis.com

What we'll discuss today...

- Any remaining questions
 - Recap what we learned
- Personal genomics
- Drug development
- Biomarker validation





Recapturing what learned

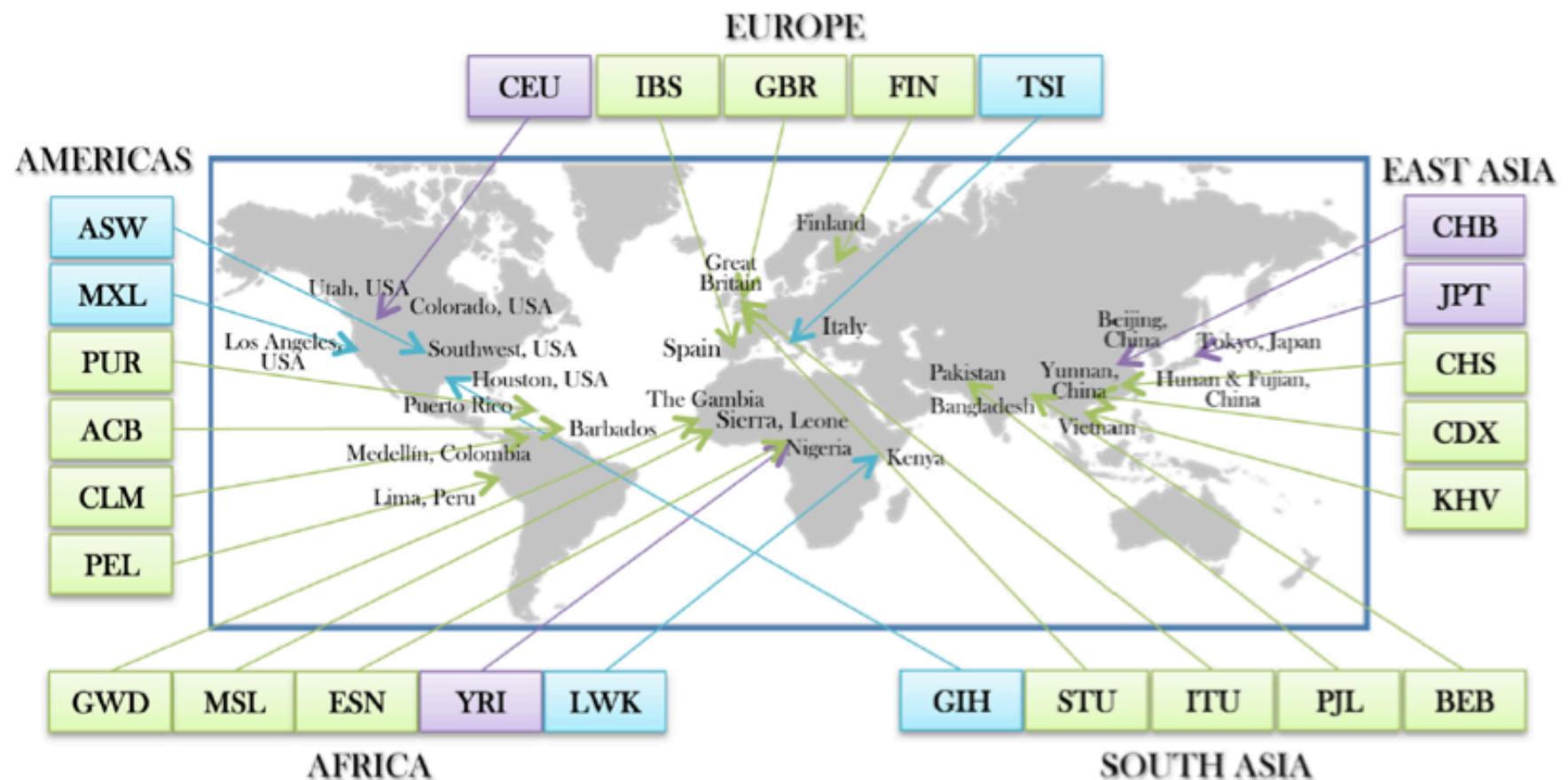
MODERN-DAY GENOMICS



UMC Utrecht



Millions of variants in many populations



International HapMap Population

HapMap 3 Population

New 1000 Genomes Population

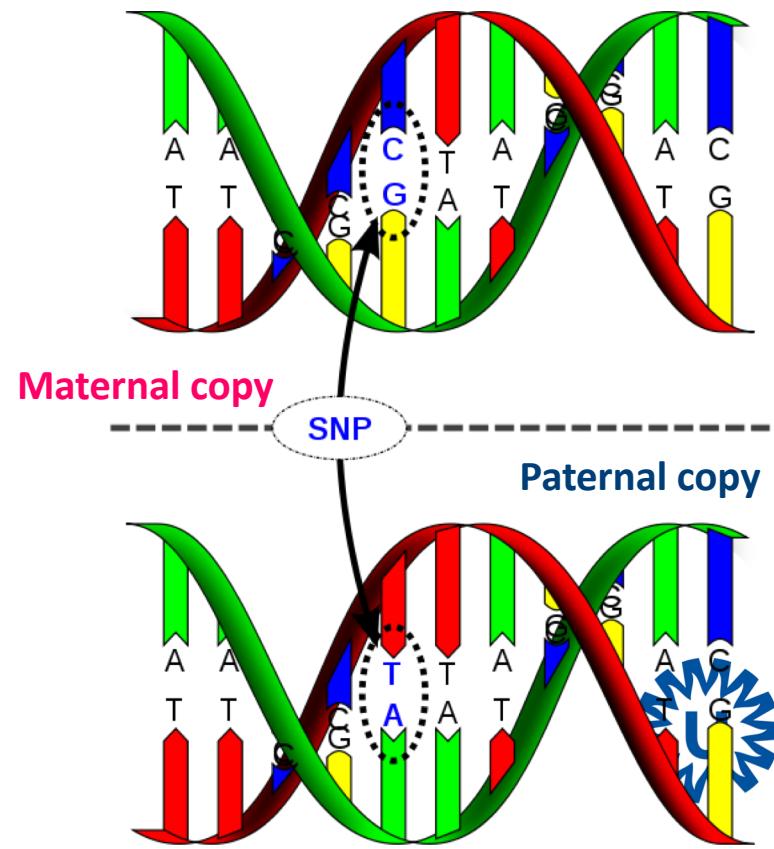


Single-Nucleotide Polymorphism

- “one base pair variation”
 - > 1% general population (common)
 - ≈10 million SNPs ($\approx 0.25\%$ genome)
 - Makes you and me unique
 - Most common type of genetic variation
- Can alter amino acid sequence
- Differential correlation between SNPs in various populations
- Used as proxies in genetic associations studies

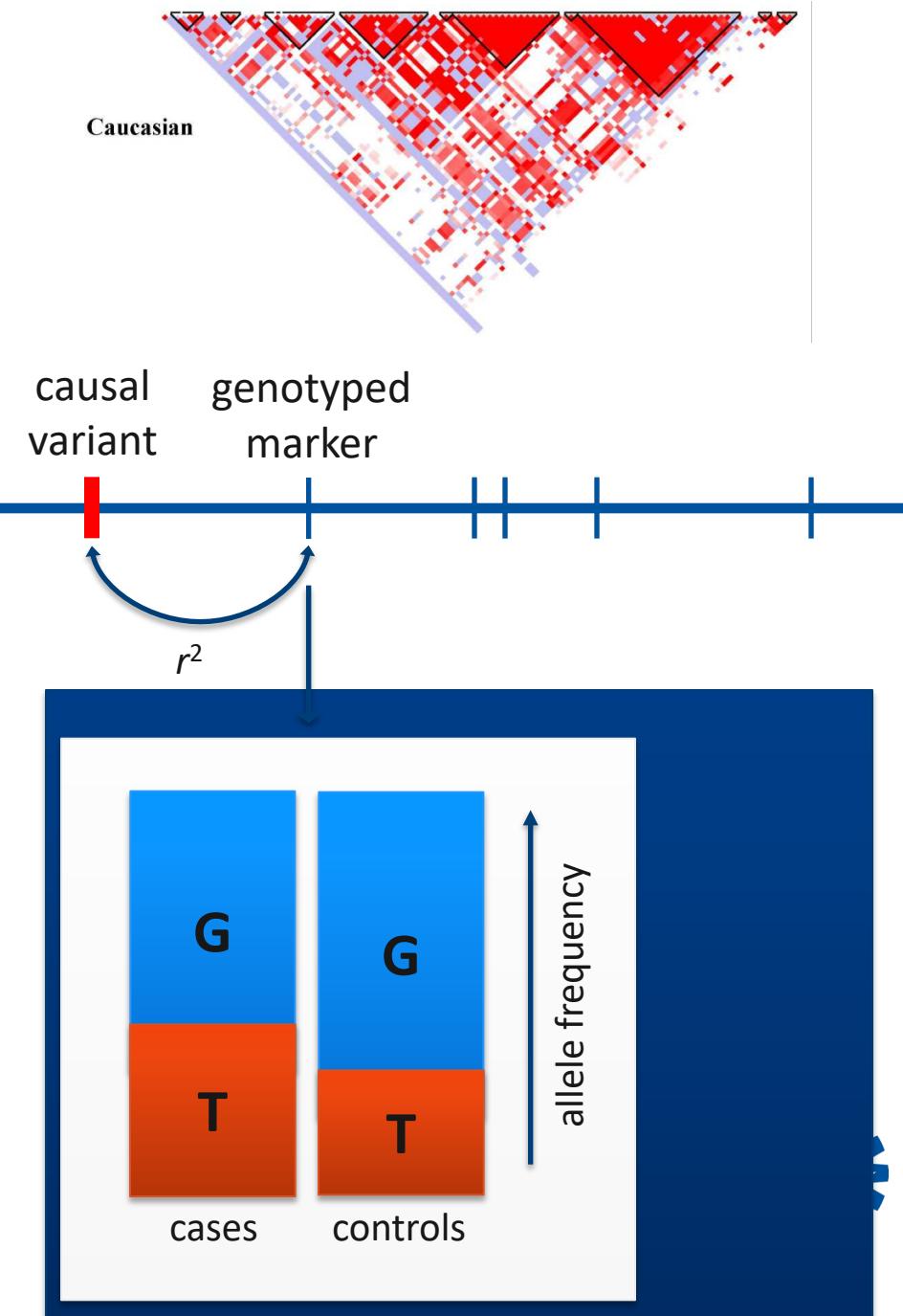


www.hapmap.org



Linkage disequilibrium

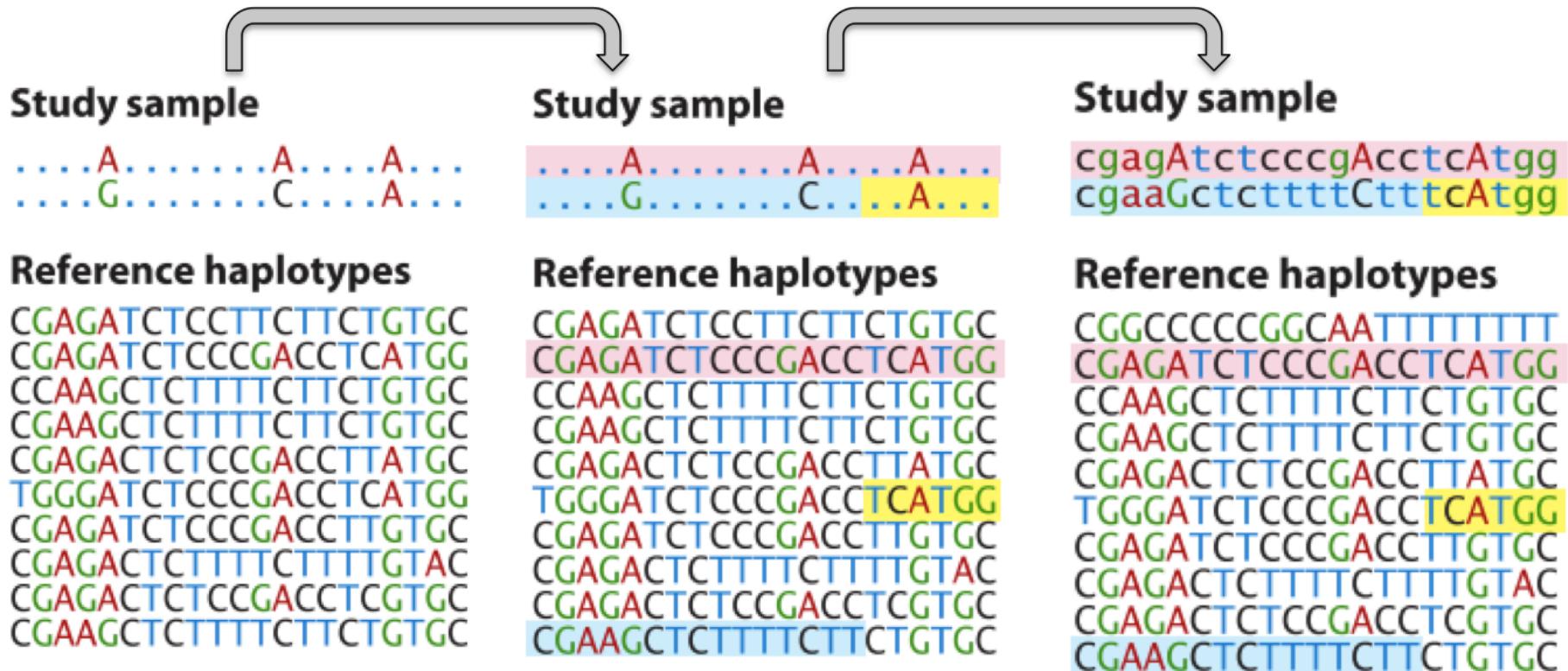
Non-random association of alleles at two or more loci

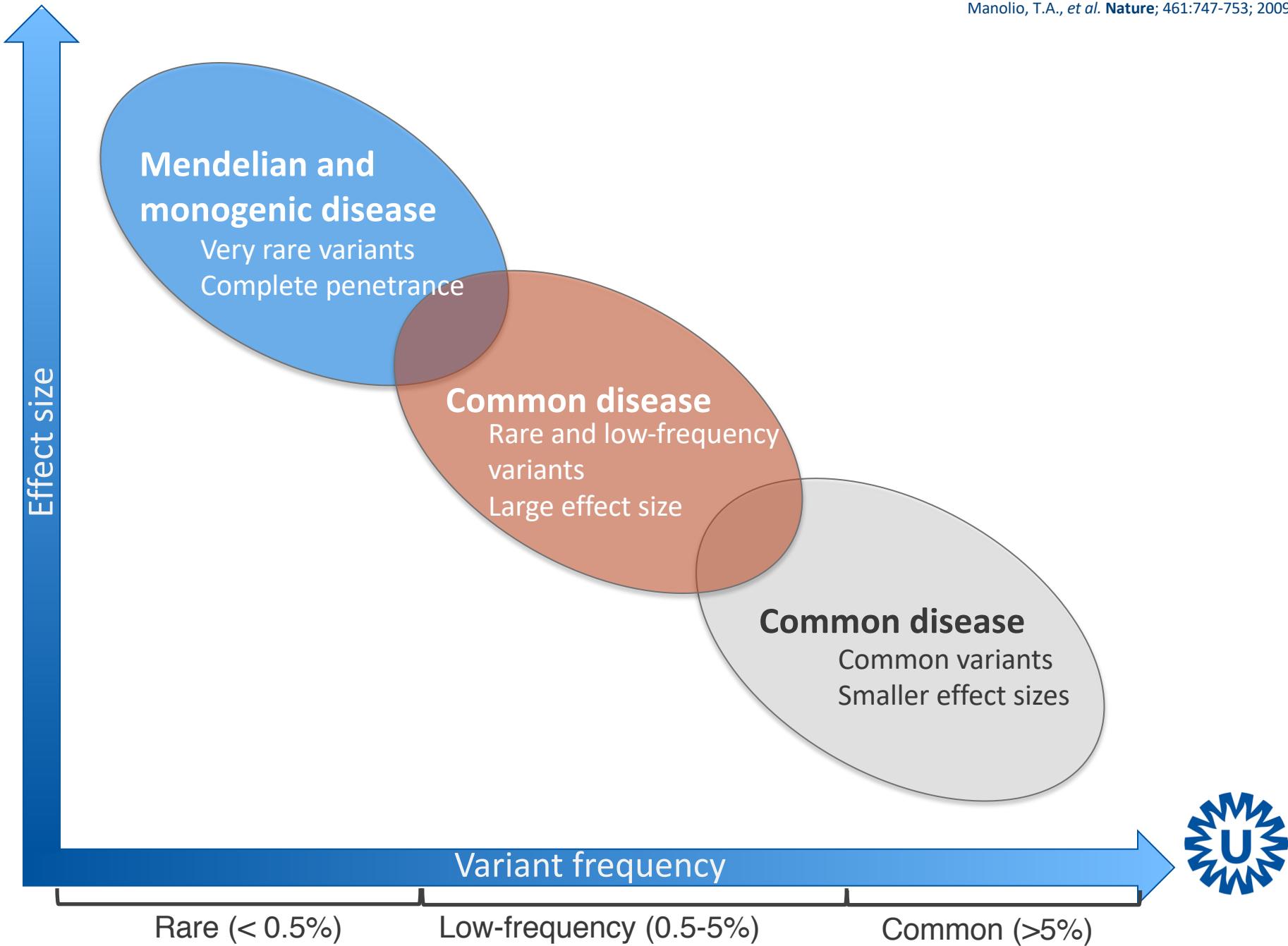


- Because of linkage disequilibrium between SNPs
- Genetic association studies can investigate genotyped markers (SNPs)
- And use these as proxies of the causal variant

Imputation

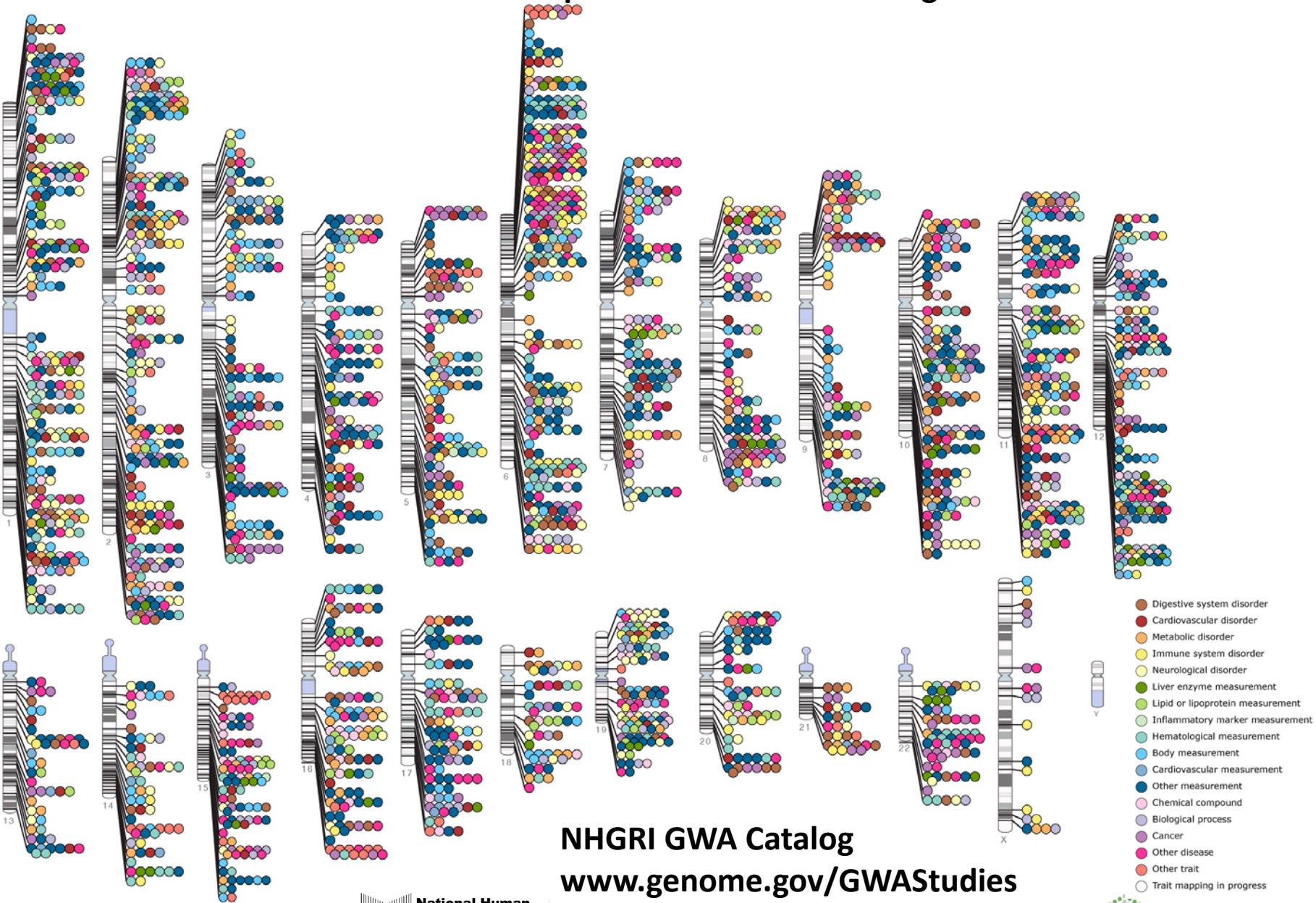
- Imputation: infer untyped genotypes based on a suitable reference panel of well-characterized and validated genotypes





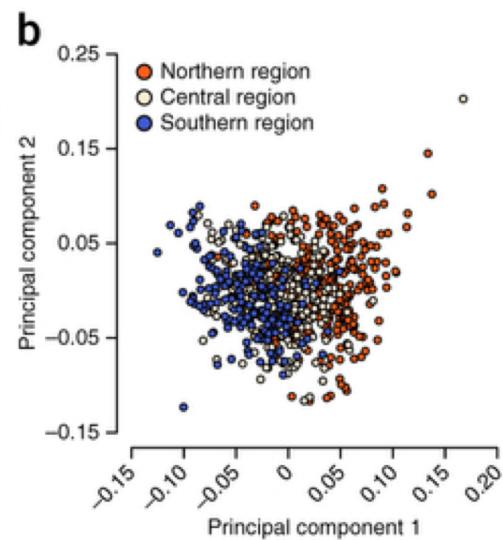
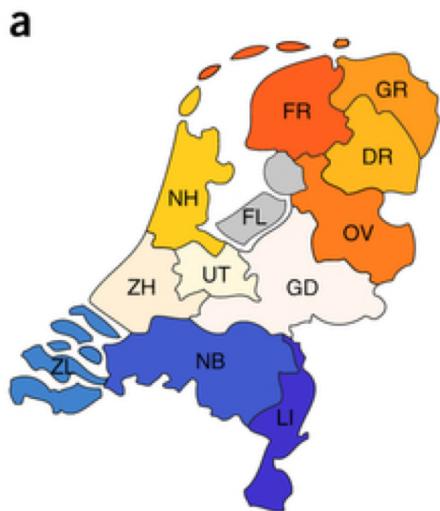
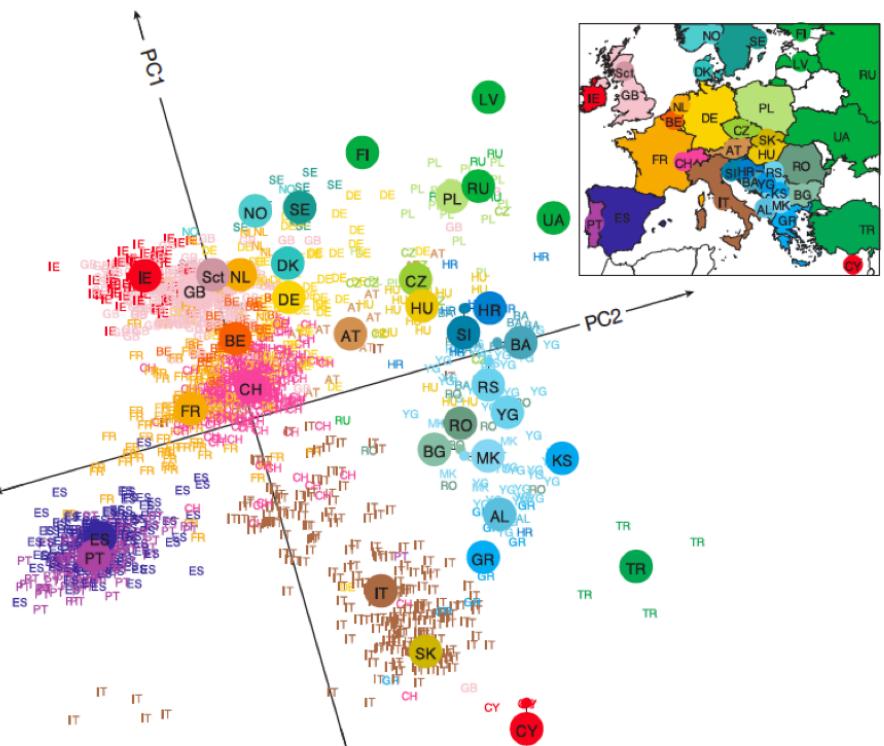
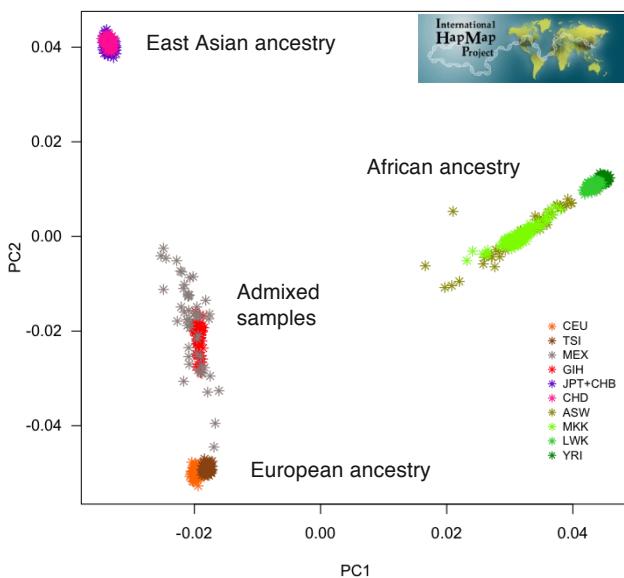
Published Genome-Wide Associations through 07/2012

Published GWA at $p \leq 5 \times 10^{-8}$ for 18 trait categories

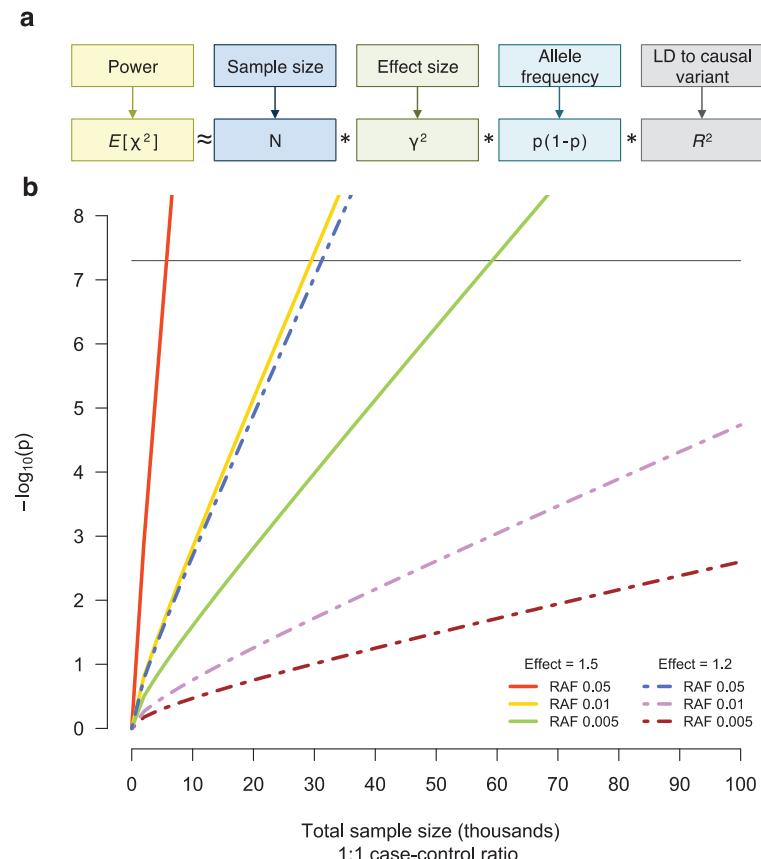
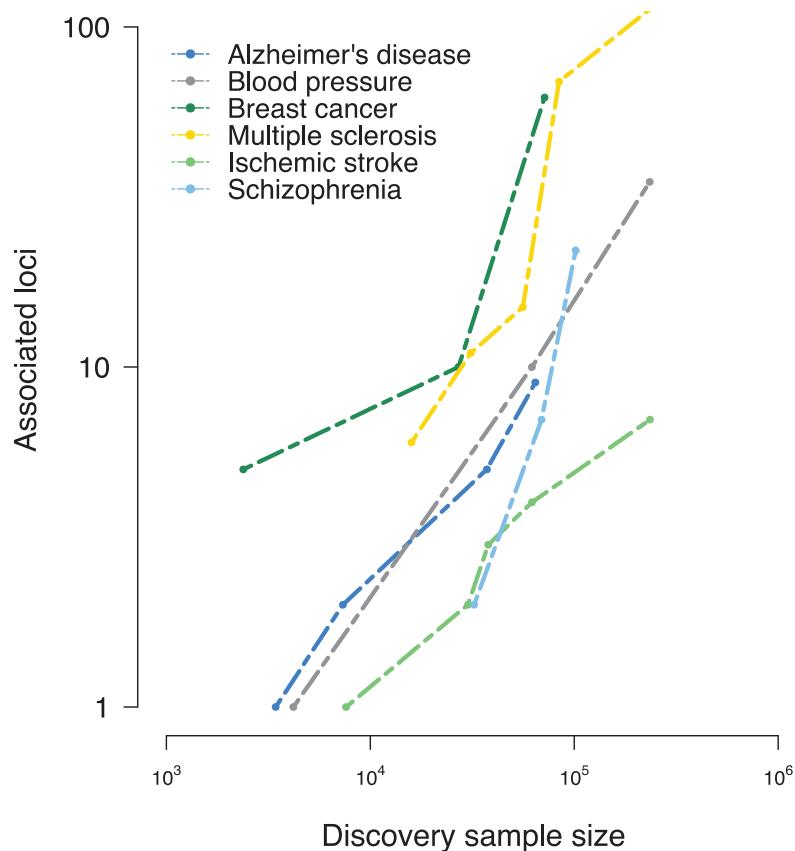


NHGRI GWA Catalog
www.genome.gov/GWASStudies
www.ebi.ac.uk/fgpt/gwas/

Population stratification



Power, Effect size, Sample size...





Clinically applying genomics

PERSONAL GENOMICS



UMC Utrecht



9p21 was used in a laboratory DNA test

- deCODE Genetics' deCODE MI™
- Assessment of the risk for (early-onset) myocardial infarction
 - SNPs rs133049 and rs10757278 located in vicinity to *CDKN2A* and *CDKN2B*
 - Risk allele is *independent* and *additive* to traditional risk factors (Framingham Heart Score, Reynold's score, AIRIC score)
 - 20-22% of the general population carry the risk allele
 - ≥40% in patients suffering early MI (male < 50 years, female < 60 years)
 - Carrying two copies of the risk allele correspond to an approximate *1.6 fold increase over the general population* of early onset MI and a *1.3 fold risk of MI in general*
- Tested according to CLIA, but not FDA approved
- Collaboration with clinicians/clinical geneticists



deCODE MI™ provided a modified 10 year CHD risk



NAME: Jane Doe DOB: 10-13-1968 GENDER: Female Patient ID: n/a deCODE ID: DGMIW#8570 5/5

FRAMINGHAM RISK SCORING ALGORITHMS FEMALE SPECIFIC

HDL - Cholesterol			Blood Pressure							
(mg/dL)	(mmol/L)	Points	Systolic		Diastolic (mmHg)			Age		
< 35	≤ 0.90	5	(mmHg)	< 80	80 - 84	85 - 89	90 - 99	≥100	Years	Points
35-44	0.91-1.16	2	< 120	-3					30-34	-9
45-49	1.17-1.29	1	120-129	0					35-39	-4
50-59	1.30-1.55	0	130-139	1					40-44	0
≥ 60	≥ 1.56	-2	140-159	2					45-49	3
			≥ 160	3					50-54	6

Note: When systolic and diastolic pressure provide different estimates for point scores, use the higher number.

LDL - Cholesterol		
(mg/dL)	(mmol/L)	Points
< 100	≤ 2.59	-2
100-129	2.60-3.36	0
130-159	3.37-4.14	0
160-189	4.15-4.91	2
≥ 190	≥ 4.92	2

Key

Color	Risk
Green	Very low
White	Low
Yellow	Moderate
Rose	High
Red	Very high

Adding up the points

Age
LDL Cholesterol
HDL Cholesterol
Blood Pressure
Diabetes
Smoker

Point total:

Comparative Risk & Your Modified Risk

Age (years)	Average 10 Year CHD Risk	Low* 10 Year CHD Risk	YOUR RISK
30-34	<1%	<1%	9%
35-39	1%	<1%	9%
40-44	2%	2%	9%
45-49	5%	3%	9%
50-54	8%	5%	9%
55-59	12%	7%	9%
60-64	12%	8%	9%
65-69	13%	8%	9%
70-74	14%	8%	9%

*Low risk was calculated for a woman the same age, normal blood pressure, LDL cholesterol 100-129 mg/dL, HDL cholesterol 45 mg/dL, non-smoker and no diabetes.

CHD Traditional Risk & Your Modified Risk			
Points Total	10 Year CHD Risk Traditional	Reclassified MI Risk Factor	10 Year CHD Risk Modified
-2	=	≤1 % x	2.35 = ≤2.4 %
-1	=	2 % x	2.35 = 4.7 %
0	=	2 % x	2.35 = 4.7 %
1	=	2 % x	2.35 = 4.7 %
2	=	3 % x	2.35 = 7.1 %
3	=	3 % x	2.35 = 7.1 %
4	=	4 % x	2.35 = 9.4 %
5	=	5 % x	2.35 = 11.8 %
6	=	6 % x	2.35 = 14.1 %
7	=	7 % x	2.35 = 16.4 %
8	=	8 % x	2.35 = 18.8 %
9	=	9 % x	2.35 = 21.2 %
10	=	11 % x	2.35 = 25.9 %
11	=	13 % x	2.35 = 30.6 %
12	=	15 % x	2.35 = 35.3 %
13	=	17 % x	2.35 = 40.0 %
14	=	20 % x	2.35 = 47.0 %
15	=	24 % x	2.35 = 56.4 %
16	=	27 % x	2.35 = 63.5 %
217	=	32 % x	2.35 = 75.2 %

In the line with your Points Total, you will find your 10 CHD Risk in the column to the right: 10 Year CHD Risk Modified. Enter YOUR 10 Year MODIFIED CHD Risk on the appropriate age group line in the Comparative Risk table to see how your risk compares to the average and low 10 year CHD risk.

This test was developed and its performance characteristics determined by the deCODE genetics Diagnostic Laboratory. It has not been cleared or approved by the U. S. Food and Drug Administration (FDA) deCODE Diagnostics Laboratory – Testing Site: Sturbridge, MA 01582, USA Phone: (877) 222-8510 Fax: (830) 785-0998 • www.decodediagnostics.com Customer Service: 15700 W. 103rd St., Suite 200, Lenexa, KS 66219 • Phone: (877) 222-8510 Fax: (830) 785-0998 • www.decodediagnostics.com

©2009 deCODE genetics Diagnostic Laboratory All rights reserved – document version 2.1

CHD Traditional Risk & Your Modified Risk

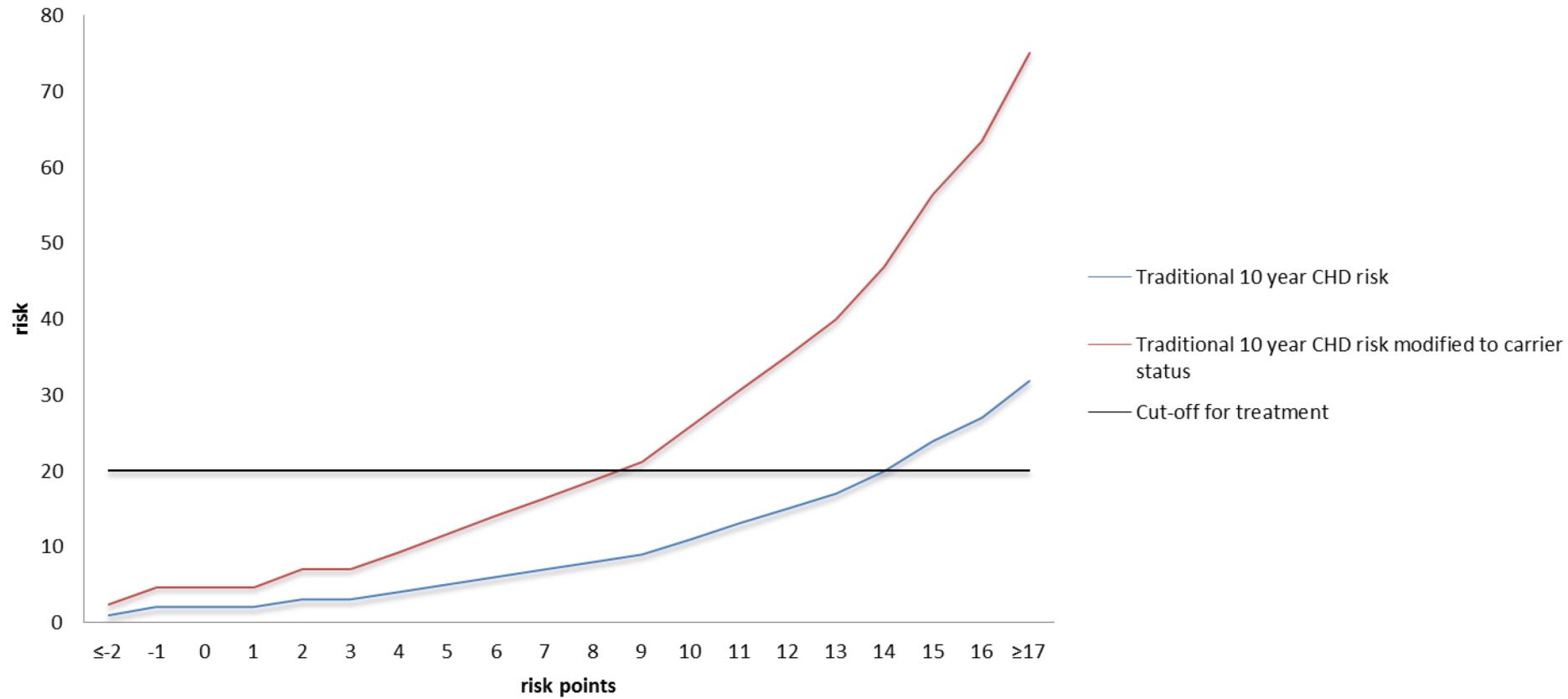
Points Total	10 Year CHD Risk Traditional	Reclassified MI Risk Factor		10 Year CHD Risk Modified
		≤1 %	x	
≤-2	=	≤1 %	x	2.35 = ≤2.4 %
-1	=	2 %	x	2.35 = 4.7 %
0	=	2 %	x	2.35 = 4.7 %
1	=	2 %	x	2.35 = 4.7 %
2	=	3 %	x	2.35 = 7.1 %
3	=	3 %	x	2.35 = 7.1 %
4	=	4 %	x	2.35 = 9.4 %
5	=	5 %	x	2.35 = 11.8 %
6	=	6 %	x	2.35 = 14.1 %
7	=	7 %	x	2.35 = 16.4 %
8	=	8 %	x	2.35 = 18.8 %
9	=	9 %	x	2.35 = 21.2 %
10	=	11 %	x	2.35 = 25.9 %
11	=	13 %	x	2.35 = 30.6 %
12	=	15 %	x	2.35 = 35.3 %
13	=	17 %	x	2.35 = 40.0 %
14	=	20 %	x	2.35 = 47.0 %
15	=	24 %	x	2.35 = 56.4 %
16	=	27 %	x	2.35 = 63.5 %
≥17	≥32 %	≥32 %	x	2.35 = ≥75.2 %

Cut-off, at which point some form of (drug) therapy starts



From 9 to more than 20% risk

10 year CHD risk modified by carrier status



So what happened to deCODE Genetics?

The screenshot shows a news article from The Wall Street Journal's Business section. The headline reads "Genomics Pioneer DeCODE Seeks Bankruptcy Protection". Below the headline, there is a sidebar for "TOP STORIES IN BUSINESS" featuring stories about Christie's CEO, Sprint, Facebook, and GM. There are also social sharing buttons for Email, Print, and 1 Comment, along with links to Facebook, Twitter, and LinkedIn.

HEALTH

Genomics Pioneer DeCODE Seeks Bankruptcy Protection

By JEANNE WHALEN

Updated Nov. 18, 2009 12:01 a.m. ET
DeCODE genetics Inc., a pioneer in the study of the human genome, filed for bankruptcy protection after struggling for years to capitalize on the relatively new field of genomics.

DeCODE, based in Reykjavik, Iceland, has sought to unlock genetic secrets about diseases such as cancer and schizophrenia by studying the relatively homogeneous gene pool of its isolated home country.

The screenshot shows a news article from Reuters. The headline is "UPDATE 3-Icelandic gene pioneer Decode files for bankruptcy". Below the headline, there is a sidebar for "RELATED NEWS" featuring stories about Lehman, UPDATE 3-Champion, and UPDATE 2-Simmons. There are also social sharing buttons for Twitter, LinkedIn, Facebook, Email, and Print.

UPDATE 3-Icelandic gene pioneer Decode files for bankruptcy

Tue Nov 17, 2009 2:56pm EST

[Tweet](#) 0 [Share](#) [Share this](#) 8+ 0 [Email](#) [Print](#)

RELATED NEWS

Lehman sues Barclays over windfall profits

UPDATE 3-Champion seeks Chapter 11 for U.S. operations

UPDATE 2-Simmons mattress company is bankrupt, to be sold

* To sell substantially all of its assets

* Lists assets of \$69.9 million, debt of \$313.9 million

* Shares plunge around 83 percent (Recasts with CEO interview, share drop, background)

By Ben Hirschler

TRENDING ON REUTERS

Boehner proposes long-term spending bill to avoid shutdown fight: aide 1

Exclusive: FBI warns of 'destructive' malware in wake of Sony attack 2

Lebanon detains wife of Islamic State leader 3

Global shares take heart from oil bounce, dollar capped 4

Congressional aide resigns after slamming Obama daughters 5

GENOMICS

Big biotech buys iconic genetics firm

Amgen's deal with deCODE Genetics shows value of combined medical and genetic data.

BY MONYA BAKER

More than a decade after the sizzle of genomics investments turned to a fizzle, a biotechnology giant is buying one of the field's flagship firms. The announcement on 10 December that Amgen, based in Thousand Oaks, California, will pay US\$415 million for Iceland's deCODE Genetics, a company with a rocky history, shows that drug developers are again ready to invest in genetic data to find therapeutic targets.

When genomics largely failed to deliver on the promise of new drug targets during the 2000s, many investors, including large drug firms, shed their stakes in companies that dealt with disease-related data and shifted to those with actual compounds in clinical trials. But data and analysis are precisely what deCODE Genetics, based in Reykjavik, has to offer. It has no drug candidates in active clinical trials, and is known chiefly for producing a steady flow of publications that pinpoint genetic culprits — and possible disease mechanisms — for conditions including neurodegeneration, cancer, cardiovascular disease and psychosis. For example, deCODE scientists this summer identified a genetic variant that protects against Alzheimer's disease (T. Jonsson *et al.*, *Nature* **488**, 96–99; 2012).

The key to deCODE's scientific success is its access to a trove of genetic data, genealogies and medical records from some 140,000 Icelanders — roughly half of Iceland's population. Being able to correlate genetic information with Iceland's extensive medical records is one reason why deCODE has made discoveries that other firms have not, says John Bell, a geneticist at the University of Oxford, UK, who chairs a UK advisory group on human genomics. "The genetics bit is the easiest bit; it's the clinical data that has historically been the problem," he says.

The rise of low-cost sequencing and electronic medical records is allowing more information to be extracted from population-genetics studies, making genomics more attractive to drug companies. Indeed, deCODE's data, technology and expertise will help Amgen to identify which experimental drugs will be most likely to succeed, says Sean Harper, head of research and development at Amgen. Already, two of Amgen's drug candidates — for heart disease and osteoporosis — take aim at protein



TO THE BRINK AND BACK

Rescued from bankruptcy, Iceland's deCODE Genetics has found new life.

1996 deCODE Genetics founded by Kári Stefánsson (pictured).

2000 deCODE goes public, raising US\$173 million.

2004–05 deCODE announces clinical trials of drugs to prevent heart attacks and asthma.

2009 deCODE declares bankruptcy.

2010 deCODE bought by some of its original investors for \$14 million.

2012 Amgen announces it will acquire deCODE.

to the Vikings, left a professorship at Harvard Medical School in Boston, Massachusetts, and returned to his homeland to found deCODE in 1996. Like other companies from the genomics boom of the early 2000s, deCODE turned to drug development as a potential source of revenue, launching its own clinical trials in the mid-2000s (see 'To the brink and back'). But the small firm soon racked up huge debts and failed to win investors as Iceland was plunged into the 2008 financial crisis. The company declared bankruptcy in 2009 and was rescued by some of its original investors, who acquired the firm and its assets for around \$14 million and went on to invest another roughly \$45 million. "Even though we are [venture capitalists], it's not just about financial returns," says Terry McGuire, a co-founder of Polaris Venture Partners in Waltham, Massachusetts. "We decided it really was a world treasure."

After the deCODE acquisition, due to be finalized in the next two weeks, Stefánsson will stay on as president of deCODE and become a vice-president at Amgen. He says that deCODE will have no lay-offs and will probably even hire staff. Stefánsson emphasizes that the new ownership will not affect how Icelanders' data are managed. The DNA samples will remain in Iceland and access will be through deCODE, subject to existing privacy-protection policies and oversight by an ethics committee of Icelandic citizens.

Scientists from deCODE are some of the most-cited geneticists, and some researchers worry that as the Icelandic group boosts Amgen's product pipelines, deCODE's leadership in basic research will fade. "deCODE may find it far harder to continue publishing its findings if they're being treated as a potential competitive advantage," says Daniel MacArthur, a geneticist at Massachusetts General Hospital in Boston.

But Harper dismisses such concerns, saying that it is in Amgen's interest to keep deCODE's scientific edge razor sharp. "For us to in some way squelch that creativity would be very short-sighted." Stefánsson is confident that business at deCODE will continue as usual. "I am unafraid, unconcerned and enthusiastic about what is happening," he says. "We will continue to indulge in our discovery orgy." ■

© NATURE.COM
The Encyclopedia of
DNA Elements:
nature.com/encode

- **AMGEN revenue**
\$18.8 billion
- **12th in the world**
after Pfizer (\$40b),
GSK (\$33.3b) and
Lilly (\$20.9b)



What is 23andMe?

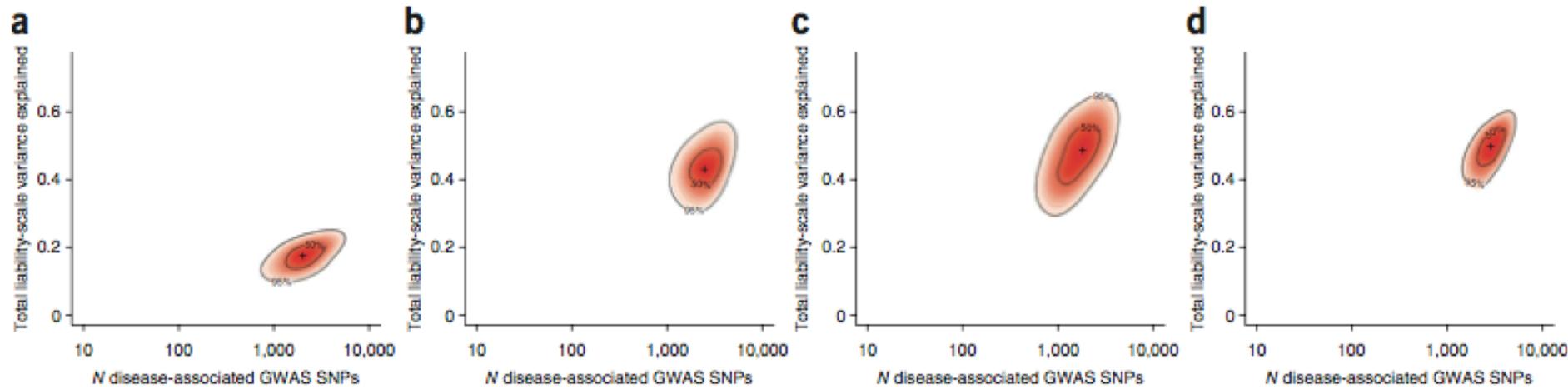
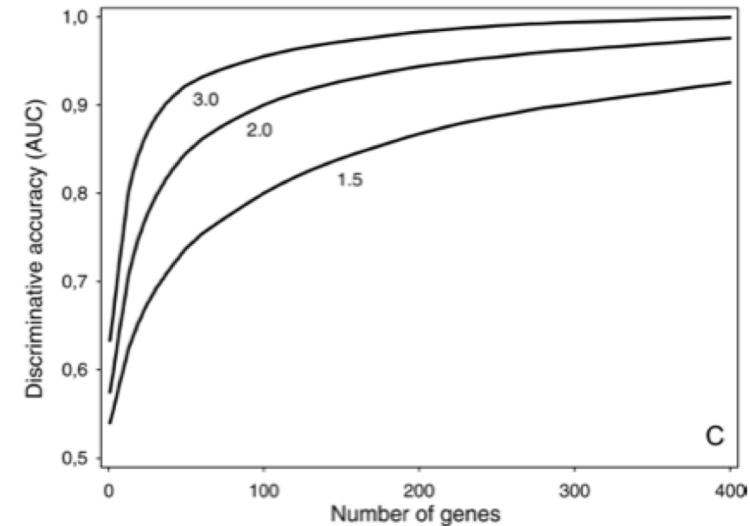


- Google “owned”
 - Google founders invested in excess of \$3.9 million
 - 23andMe raised in excess of \$50 million
-
- Personal genomics
 - Genetic information based on personal genotyping and current literature knowledge
 - Individual prediction of risk similar to deCODEme
 - Not FDA approved – forbidden to sell pending approval



The added value of Polygenic Burden/Risk Scores?

- Polygenic Burden/Risk Scores in addition to existing risk factor models
- Hundreds of variants are needed for any meaningful addition
- Upside: many variants remain to be discovered explaining more of the biology of phenotypes





Clinically applying genomics

NEW DRUGS



UMC Utrecht



Proprotein convertase subtilisin/kexin type 9

- Variants in PCSK9 associated with low LDL and lower risk for CHD
 - Cure?

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.

ABSTRACT

BACKGROUND

A low plasma level of low-density lipoprotein (LDL) cholesterol is associated with reduced risk of coronary heart disease (CHD), but the effect of lifelong reductions in plasma LDL cholesterol is not known. We examined the effect of DNA-sequence variations that reduce plasma levels of LDL cholesterol on the incidence of coronary events in a large population.

METHODS

We compared the incidence of CHD (myocardial infarction, fatal CHD, or coronary revascularization) over a 15-year interval in the Atherosclerosis Risk in Communities study according to the presence or absence of sequence variants in the proprotein convertase subtilisin/kexin type 9 serine protease gene (PCSK9) that are associated with reduced plasma levels of LDL cholesterol.

RESULTS

Of the 3363 black subjects examined, 2.6 percent had nonsense mutations in PCSK9; these mutations were associated with a 28 percent reduction in mean LDL cholesterol and an 88 percent reduction in the risk of CHD ($P=0.008$ for the reduction; hazard ratio, 0.11; 95 percent confidence interval, 0.02 to 0.81; $P=0.03$). Of the 9524 white subjects examined, 3.2 percent had a sequence variation in PCSK9 that was associated with a 15 percent reduction in LDL cholesterol and a 47 percent reduction in the risk of CHD (hazard ratio, 0.50; 95 percent confidence interval, 0.32 to 0.79; $P=0.003$).

CONCLUSIONS

These data indicate that moderate lifelong reduction in the plasma level of LDL cholesterol is associated with a substantial reduction in the incidence of coronary events, even in populations with a high prevalence of non-lipid-related cardiovascular risk factors.

From the Donald W. Reynolds Cardiovascular Clinical Research Center (J.C.C., H.H.H.), the Center for Human Genetics (J.C.C.), the Department of Internal Medicine (J.C.C., H.H.H.) and Molecular Genetics (H.H.H.), and the Howard Hughes Medical Institute (H.H.H.), University of Texas Southwestern Medical Center, Dallas; the Human Genetics Center and Institute of Molecular Medicine, University of Texas Health Science Center, Houston; and the Department of Medicine, University of Mississippi Medical Center, Jackson (T.H.M.). Address reprint requests to Dr. Hobbs at the Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas TX 75390-9046, or at helen.hobbs@utsouthwestern.edu.

N Engl J Med 2006;354:1264-72.
Copyright © 2006 Massachusetts Medical Society.

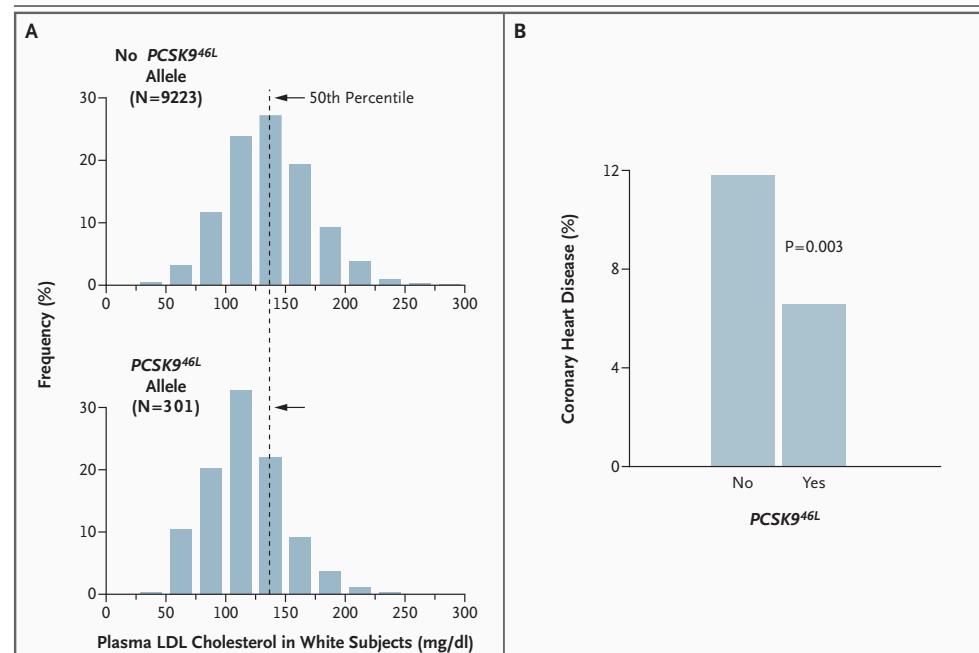


Figure 2. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Events (Panel B) among White Subjects, According to the Presence or Absence of a *PCSK9^{46L}* Allele.

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



Clinical trials for PCSK9 antibodies

Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol

Evan A. Stein, M.D., Ph.D., Scott Mellis, M.D., Ph.D.,
George D. Yancopoulos, M.D., Ph.D., Neil Stahl, Ph.D., Douglas Logan, M.D.,
William B. Smith, M.D., Eleanor Lisbon, M.D., M.P.H., Maria Gutierrez, M.D.,
Cheryle Webb, M.D., Richard Wu, Ph.D., Yunling Du, Ph.D.,
Therese Kranz, R.N., M.B.A., Evelyn Gasparino, B.S.,
and Gary D. Swerdlow, M.D., Ph.D.

ABSTRACT

BACKGROUND

Proprotein convertase subtilisin/kexin 9 (PCSK9), one of the serine proteases, binds to low-density lipoprotein (LDL) receptors, leading to their accelerated degradation and to increased LDL cholesterol levels. We report three phase 1 studies of a monoclonal antibody to PCSK9 designated as REGN727/SAR236553 (REGN727).

METHODS

In healthy volunteers, we performed two randomized, single ascending-dose studies of REGN727 administered either intravenously (40 subjects) or subcutaneously (32 subjects), as compared with placebo. These studies were followed by a randomized, placebo-controlled, multiple-dose trial in adults with heterozygous familial hypercholesterolemia who were receiving atorvastatin (21 subjects) and those with nonfamilial hypercholesterolemia who were receiving treatment with atorvastatin (30 subjects) (baseline LDL cholesterol, >100 mg per deciliter [2.6 mmol per liter]) or a modified diet alone (10 subjects) (baseline LDL cholesterol, >130 mg per deciliter [3.4 mmol per liter]). REGN727 doses of 50, 100, or 150 mg were administered subcutaneously on days 1, 29, and 43. The primary outcome for all studies was the occurrence of adverse events. The principal secondary outcome was the effect of REGN727 on the lipid profile.

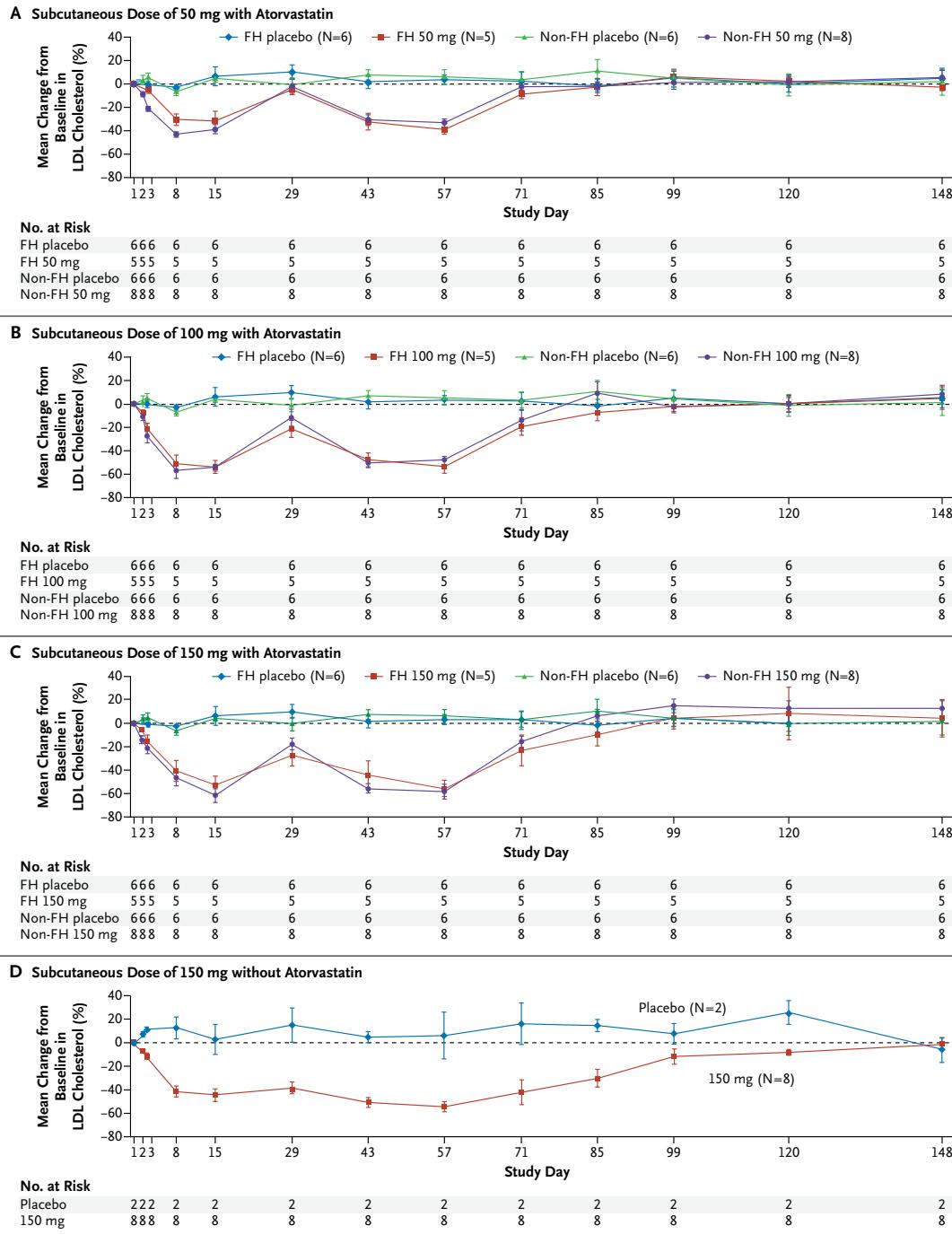
RESULTS

Among subjects receiving REGN727, there were no discontinuations because of adverse events. REGN727 significantly lowered LDL cholesterol levels in all the studies. In the multiple-dose study, REGN727 doses of 50, 100, and 150 mg reduced measured LDL cholesterol levels in the combined atorvastatin-treated populations to 77.5 mg per deciliter (2.00 mmol per liter), 61.3 mg per deciliter (1.59 mmol per liter), and 53.8 mg per deciliter (1.39 mmol per liter), for a difference in the change from baseline of -39.2, -53.7, and -61.0 percentage points, respectively, as compared with placebo ($P<0.001$ for all comparisons).

CONCLUSIONS

In three phase 1 trials, a monoclonal antibody to PCSK9 significantly reduced LDL cholesterol levels in healthy volunteers and in subjects with familial or nonfamilial hypercholesterolemia. (Funded by Regeneron Pharmaceuticals and Sanofi; ClinicalTrials.gov numbers, NCT01026597, NCT01074372, and NCT01161082.)





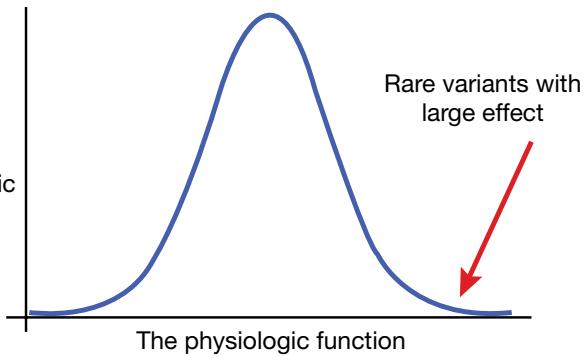
Human genetics as a foundation for innovative drug development

Alexander Kamb, Sean Harper & Kari Stefansson

New technology has transformed human genetics. It now provides perhaps the single best opportunity to innovate and improve clinical success rates in drug development.

- The human “knockout” is probably the best model

Frequency of the combination of variant alleles that impact a physiologic function



COMMENTARY

Box 1 What does genetics tell us about previous trials?

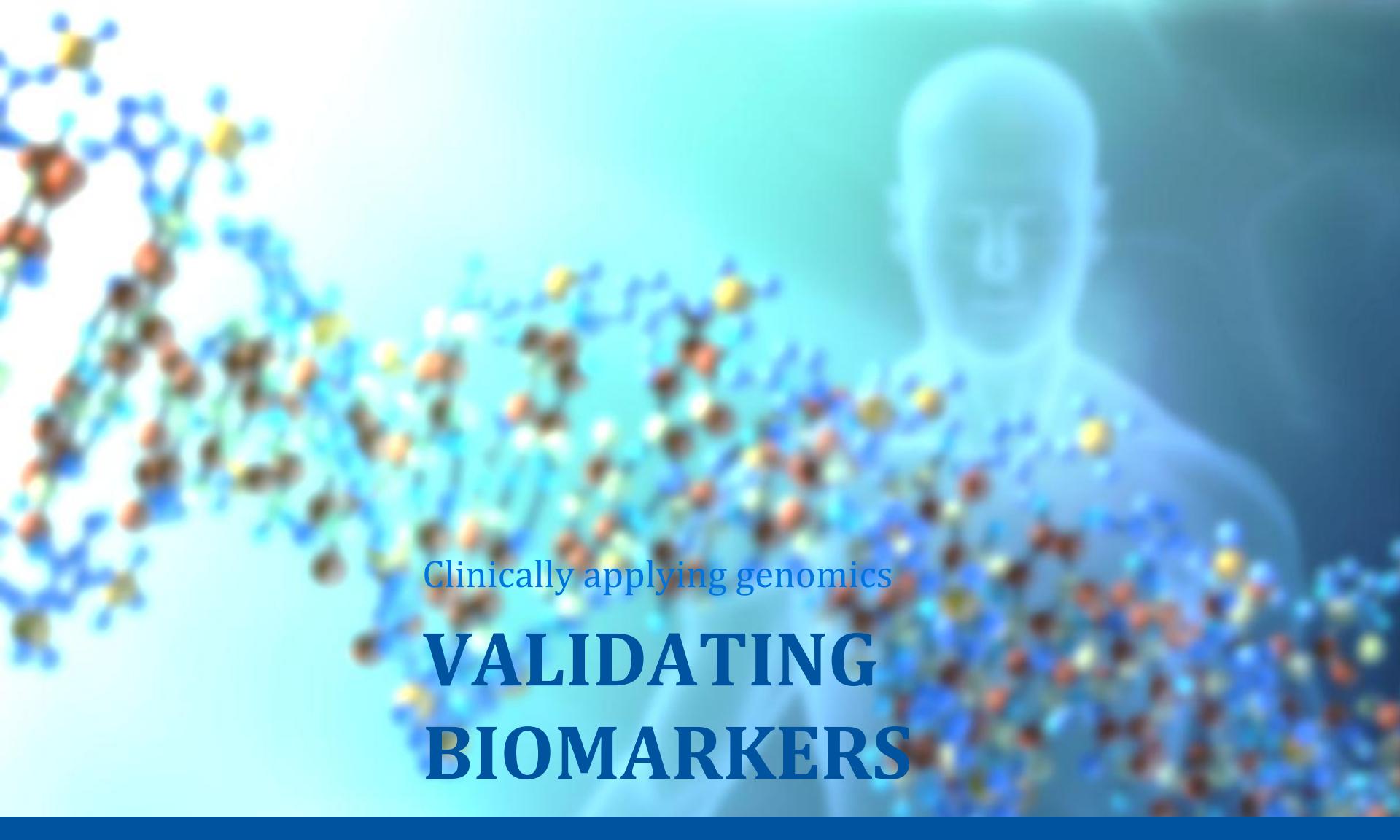
A post hoc assessment of phase 3 successes and failures (initiated 2000–2008) supports the case for genetics as a positive predictor. We used input data from business intelligence provider Informa's (Zug, Switzerland) Citeeline Pipeline (<http://www.citeeline.com/>). Cancer trials were excluded from the analysis as were vaccines, drugs of uncertain pharmacology and antisense. Trial success was defined as trials with status listed as launched, in registration or in preregistration. Trial failure was defined as trials with status listed as discontinued, no further development reported, phase 1 or phase 2 (i.e., regressed). Targets were as listed in the database and were consolidated to eliminate duplicates. If any trial of a drug against a given target succeeded, the target was listed under trial success. The genetic associations were performed for target genes

at deCODE (Reykjavik) with the trial status hidden (i.e., blinded). We tested 1,100 binary and 550 quantitative phenotypes. Criteria for association between a given marker and phenotype were amino acid-changing marker, $P < 5 \times 10^{-4}$; noncoding marker in gene vicinity, $P < 1 \times 10^{-6}$; marker previously reported in GWAS catalog, $P < 5 \times 10^{-4}$; in four additional occurrences, the result was considered significant owing to a confluence of elements. Table 1 shows the results. In the detail of the trial failures (bottom of table), it can be seen that five targets should be listed as successes, and two should be excluded based on uncertain pharmacology of the drug. Thus, all targets with clear genetic evidence and good pharmacologic agents in this set produce the clinical effect predicted by human genetics.

Table 1 Phase 3 trials of drugs against targets with genetic evidence

Drug	Primary target	Indication	deCODE phenotype ^a	P value	Odds ratio/beta	Comment
<i>Successful phase 3 trials</i>						
Fentanyl	OPRM1	Pain	Opiate use male	8E-5	0.1	Borderline significance but functional effect of variant, PMID 19528663
Metformin	PRKAA1	T2 diabetes	Type 2 diabetes	1E-8	9.8	
Tramadol	SLC6A4	Pain	Pain	5E-4	10.2	
Carvedilol	ADRB1	Hypertension	Blood pressure; systolic blood pressure	7E-4	-0.04	Replication of literature blood pressure, $P = 2E-9$, PMID 21909110
Mipomersen	APOB	Hypercholesterolemia	LDL cholesterol level	4E-37	0.1	
Gabapentin	CACNA2D1	Epilepsy	Epilepsy	5E-8	46.7	
Cinacalcet	CASR	Hypercalcemia	Calcium level	7E-37	0.1	
Varenicline	CHRNA4	Nicotine addiction	Cigarettes/day	9E-5	0.3	
Lipegfilgrastim	CSF3R	Neutropenia	Neutrophil count	5E-14	0.03	
Sitagliptin	DPP4	Type 2 diabetes	Type 1 diabetes	1E-5	2.2	Authors have validated that association is really for type 1 diabetes
Ambrisentan	EDNRA	Hypertension	Coronary artery disease before 76	6.8E-05	1.11	Variant reported for carotid intima media thickness, $P = 7E-12$, PMID 21909108
Lasofoxifene	ESR1	Osteoporosis	Bone mineral density	5E-17	0.1	
Somatropin	GHR	Dwarfism	Height	3E-12	-0.26	
Simvastatin	HMGCR	Hypercholesterolemia	LDL cholesterol level	3E-29	0.08	
Ustekinumab	IL12B	Psoriasis	Psoriasis	1E-16	1.41	
Afamelanotide	MC1R	Sun-induced skin disorders	Sun sensitivity	3E-84	2.3	
Esetimibe	NPC1L1	Hypercholesterolemia	LDL cholesterol level	5E-9	0.05	
Fenofibrate	PPARA	Atherosclerosis	LDL cholesterol level	2E-29	0.08	
Adalimumab	TNF	Rheumatoid arthritis	Rheumatoid arthritis	7E-38	2.04	Association points to human leukocyte antigen (HLA) region where tumor necrosis factor alpha (TNF α) nested-HLA genes are classically discussed; however, other genes cannot be excluded
Denosumab	TNFSF11	Osteoporosis	Bone mineral density	9E-29	0.14	
Pegaptanib	VEGFA	Macular degeneration	Macular degeneration	6E-5	1.2	
<i>Phase 3 trial ‘failures’</i>						
Torcetrapib	CETP	Hypercholesterolemia	HDL cholesterol level	8E-181	0.22	HDL endpoint met, not outcome
Roxithromycin	ITGA2B	Thrombosis	Platelet count	1E-6	-0.21	Injected drug approved, not oral
Tedisamil	KCHN2	Atrial fibrillation	QT interval	6E-8	-0.05	Efficacy for atrial arrhythmia, but risk at high doses—not specific for this K $^{+}$ channel
Liprotamase	PNLIP/AMY2A	Pancreatic insufficiency	Lipase/amylase level	2E-64/ 1E-145	-0.16/ -0.28	Head to head with porcine product needed for approval
Rosiglitazone	PPARG	Type 2 diabetes	Type 2 diabetes	4E-4	0.8	Glitzones already approved for type 2 diabetes; combo tested in this study
Tagatose	PYGL	Type 2 diabetes	Type 2 diabetes	7E-7	4.0	Small effect, but tagatose (sugar) is a very weak inhibitor

^a‘deCODE Phenotype’ refers to trait or diagnosis within the deCODE Genetics’ phenotype database.



Clinically applying genomics

VALIDATING BIOMARKERS



UMC Utrecht



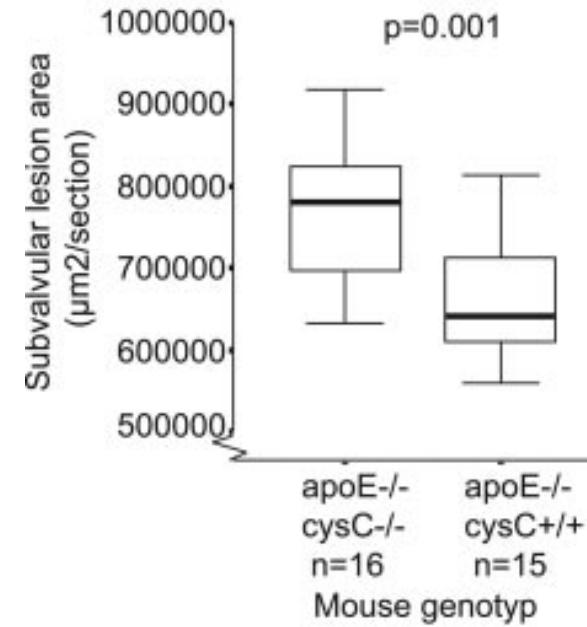
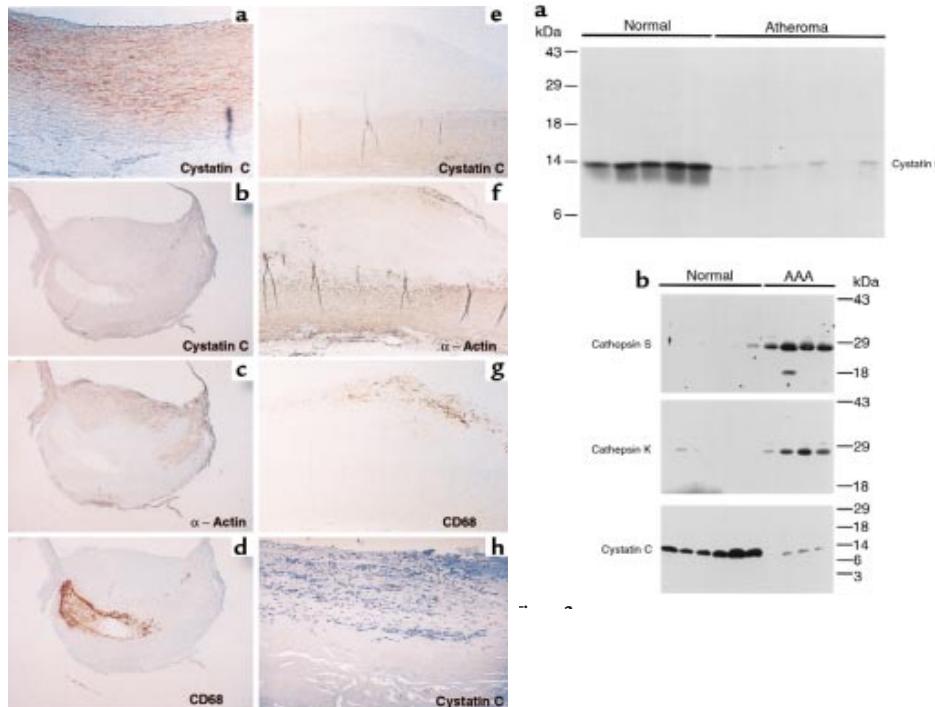
Cystatin C

- Encoded by *CST3* on 20p11.21
- Protease inhibitor
 - Activity of cathepsins (cysteine proteases) in vascular smooth muscle cells
 - Prevention of plaque formation by binding amyloid-beta precursor protein (APP) in Alzheimer's disease



A role in atherosclerotic disease

- Reduction of Cystatin C in abdominal aortic aneurysm (in smooth muscle cells)
- Lack of Cystatin C in mice promotes atherosclerosis



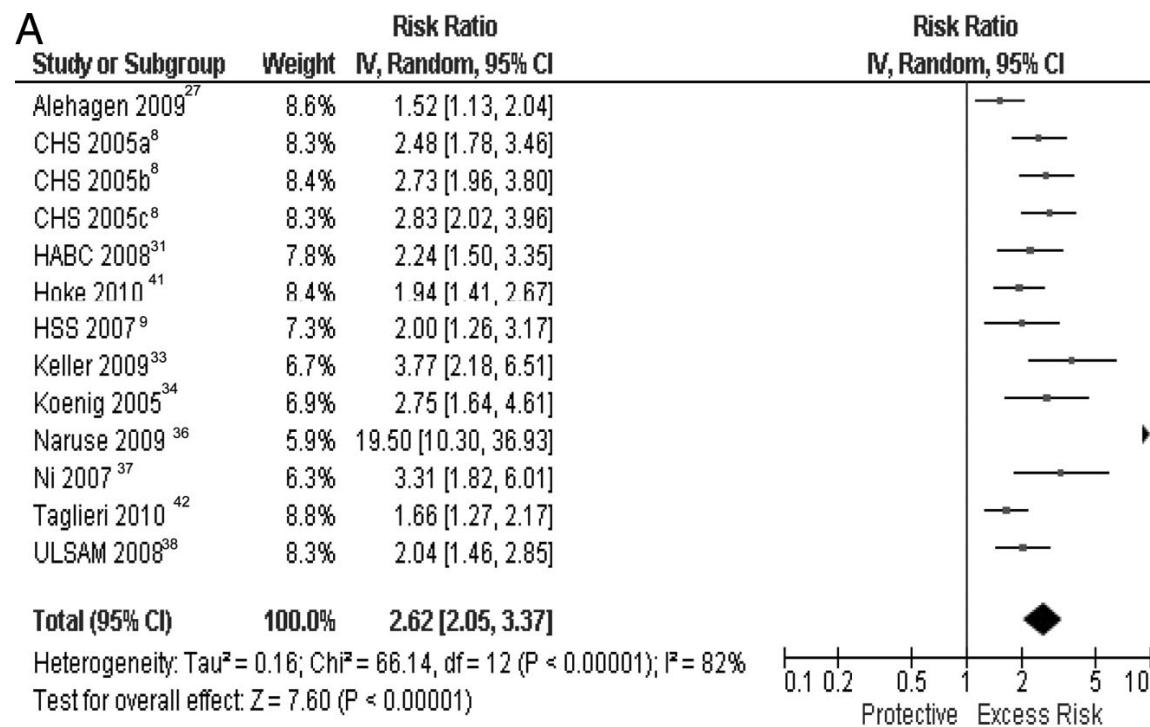
Epidemiological evidence

- In elderly without chronic kidney disease (CKD), Cystatin C (CystC) is prognostic of death, cardiovascular disease (CVD) and CKD
- Asymptomatic carotid atherosclerosis patients, CystC prognostic for CVD
- CystC associated with lesion size in acute stroke



Literature meta-analyzed

- Cystatin C is associated with a higher risk of CVD, stroke and mortality in high-risk populations
 - With heart failure, CHD, >70 years...



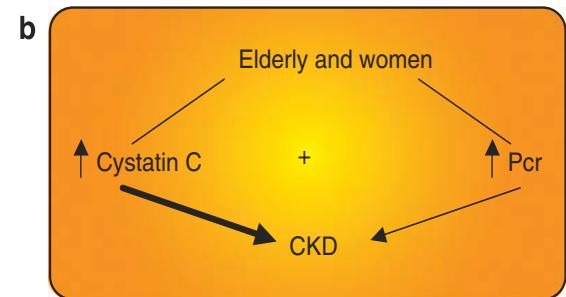
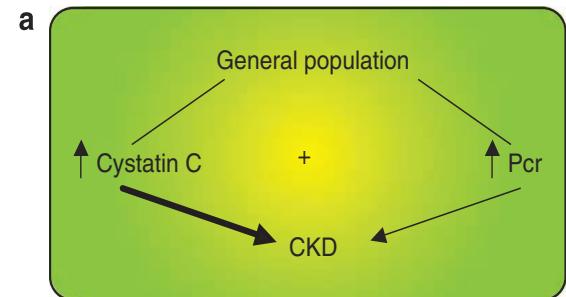
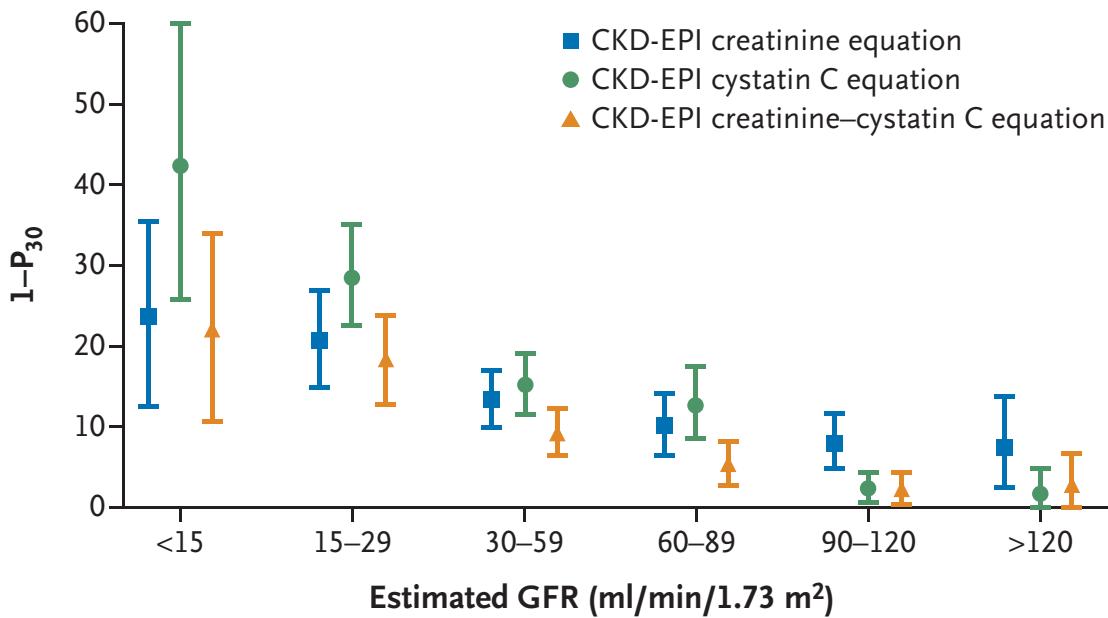


CHRONIC KIDNEY DISEASE



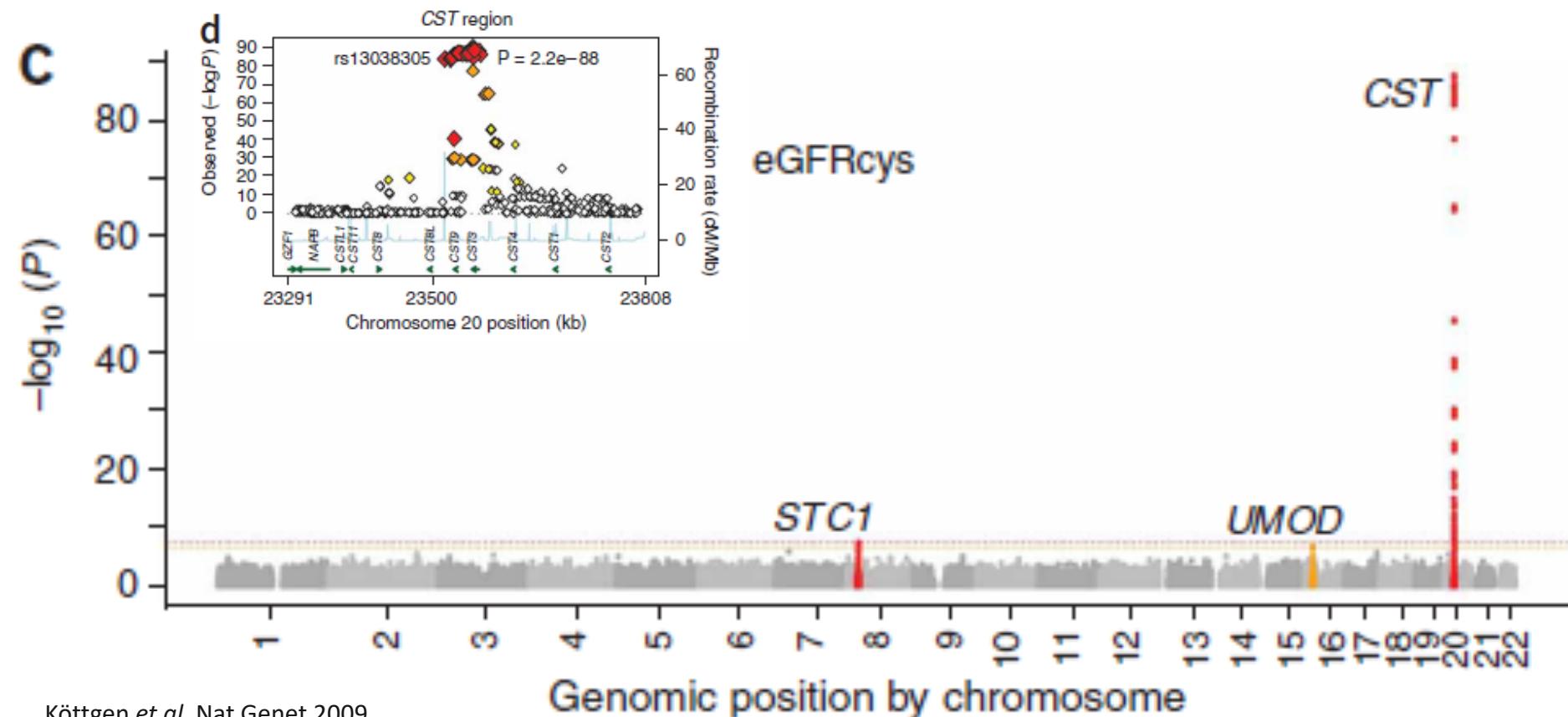
Cystatin C as a biomarker for CKD

- One of the best naturally occurring biomarker for human estimated glomerular filtration rate
- It is a better biomarker than creatinine when eGFR>90 (mL/min/1.73m³)



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

- Three 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 cystatin C expression: STC1, UMOD, CST3



Köttgen et al. Nat Genet 2009

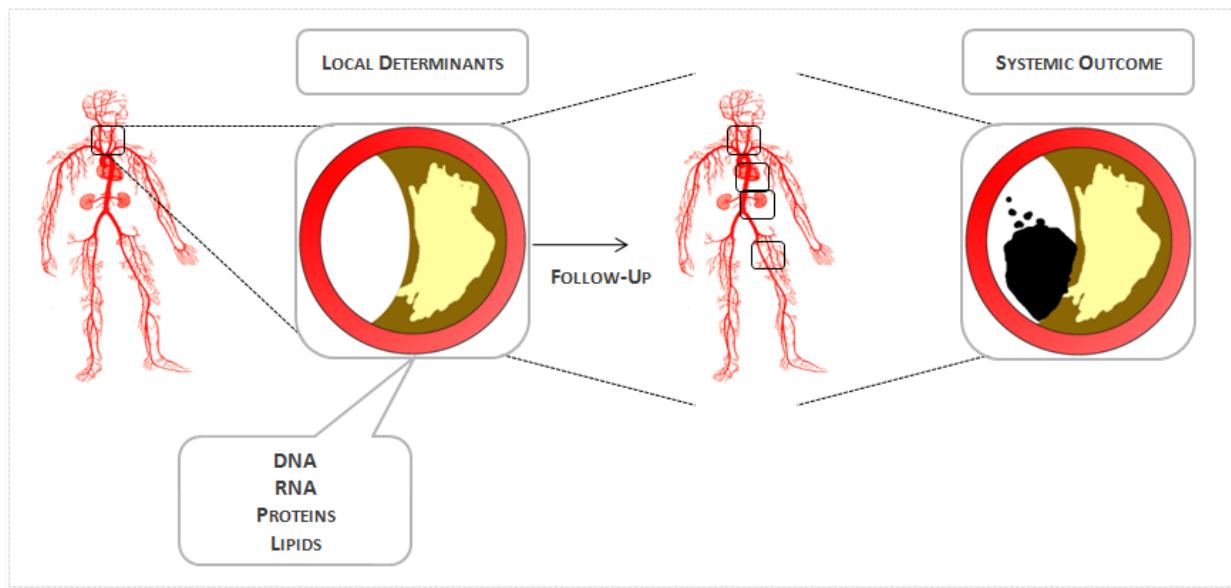
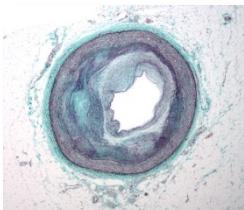


ATHERO-EXPRESS



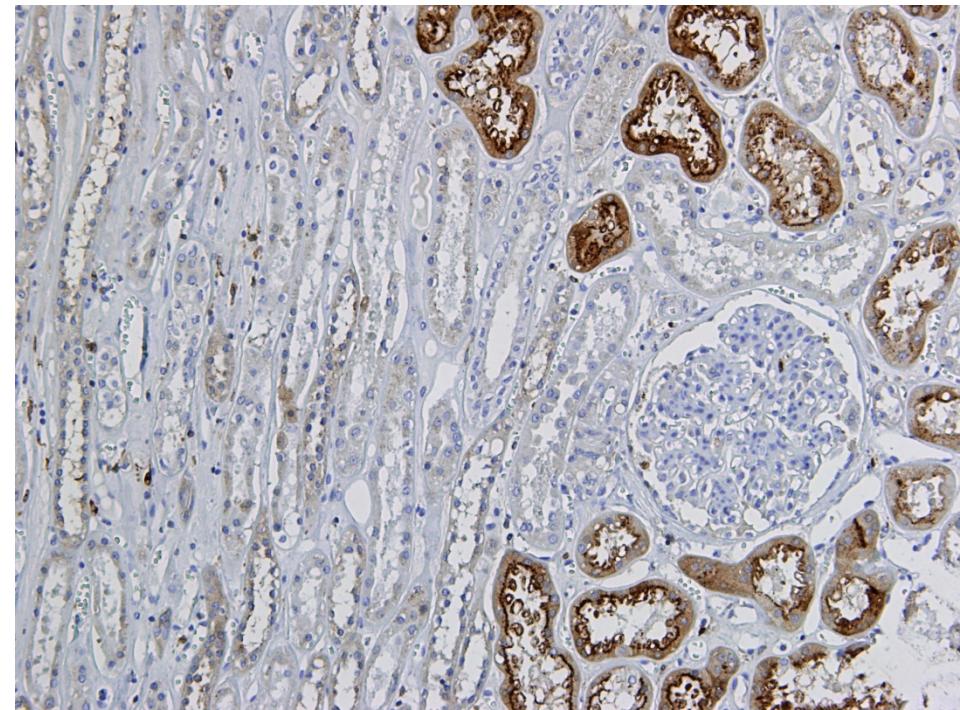
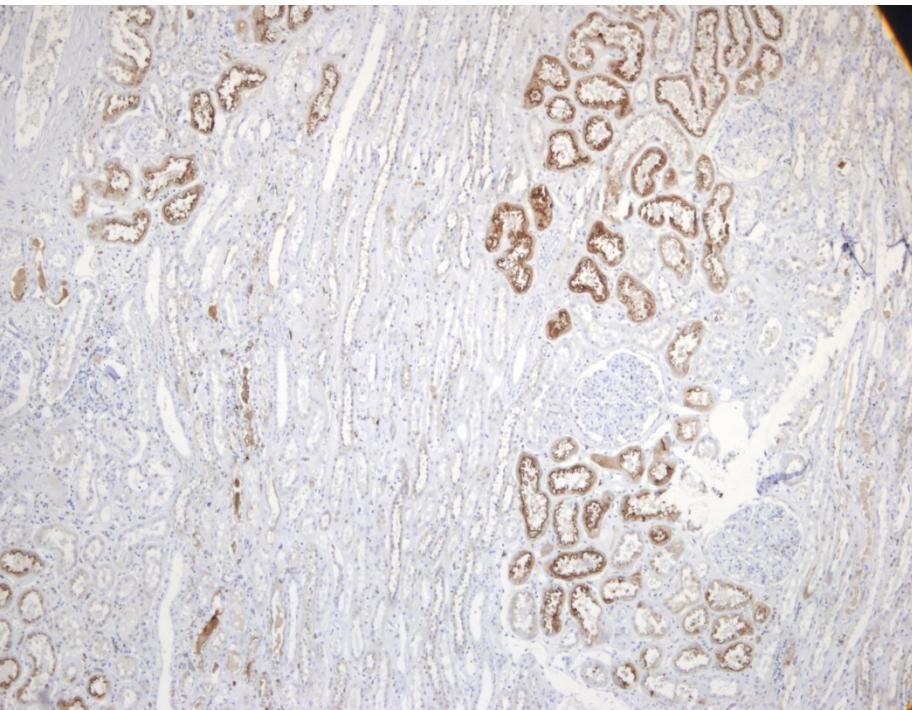
This was the start of our work...

- Is there an association of CystC associated variant with CystC and smooth muscle cells in the carotid plaque?



Histological staining for CystC

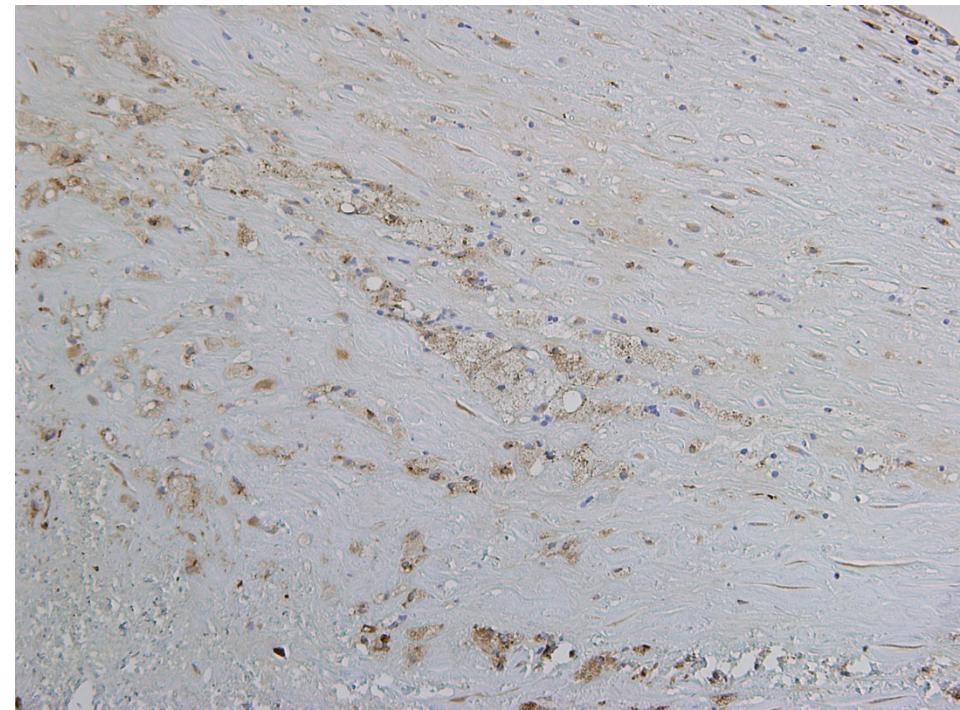
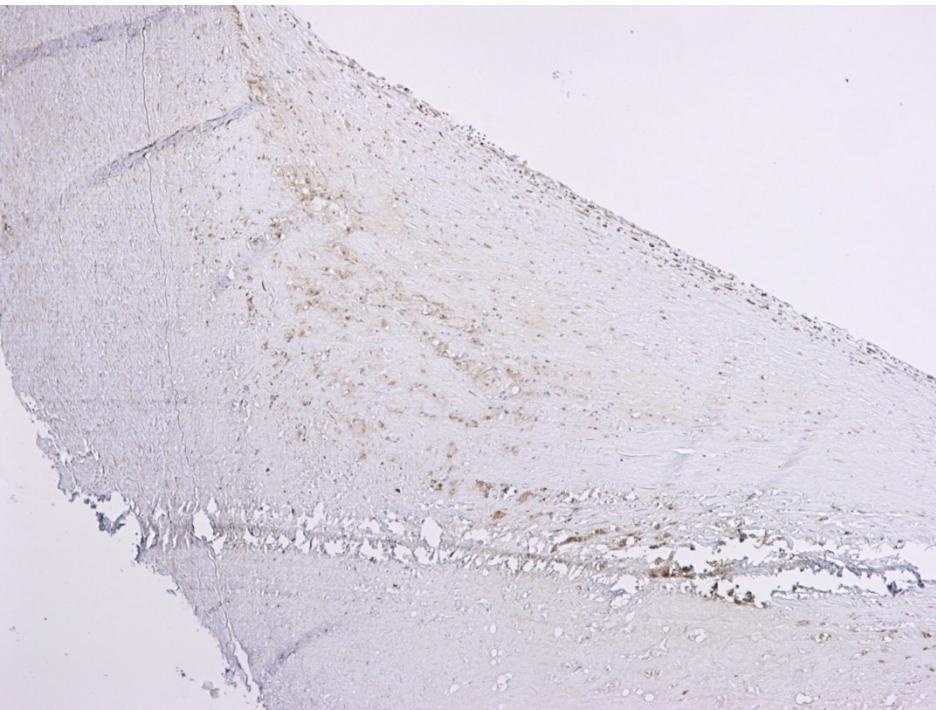
- First a positive control: the human kidney
- CystC present in proximal (?) tubuli
- Appears to be present in granules



Van der Laan *et al.* unpublished

Carotid endarterectomy sample

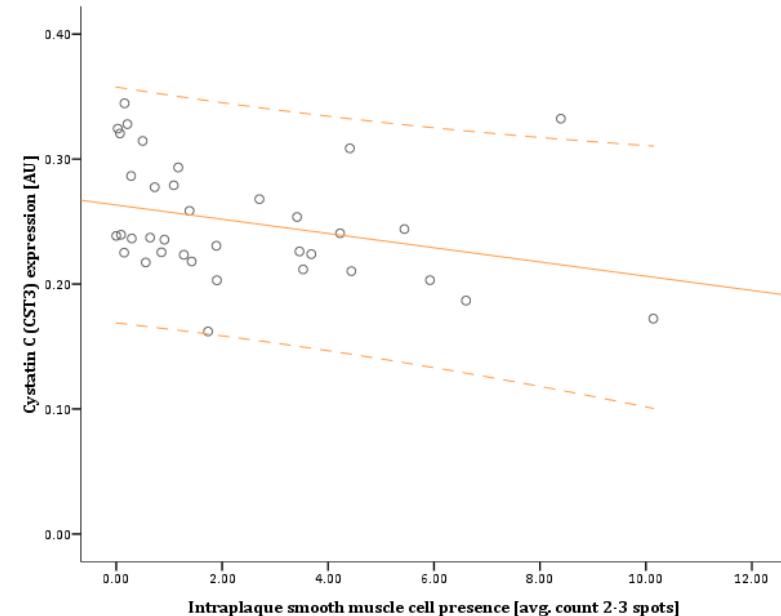
- Shoulder region, clear staining for CystC
- Appears to be stronger in foamy cells, (foamy) smooth muscle cells
- Also α -specific (?) coloring of fat
- Limitation: no double-staining for α -actin



Van der Laan *et al.* unpublished

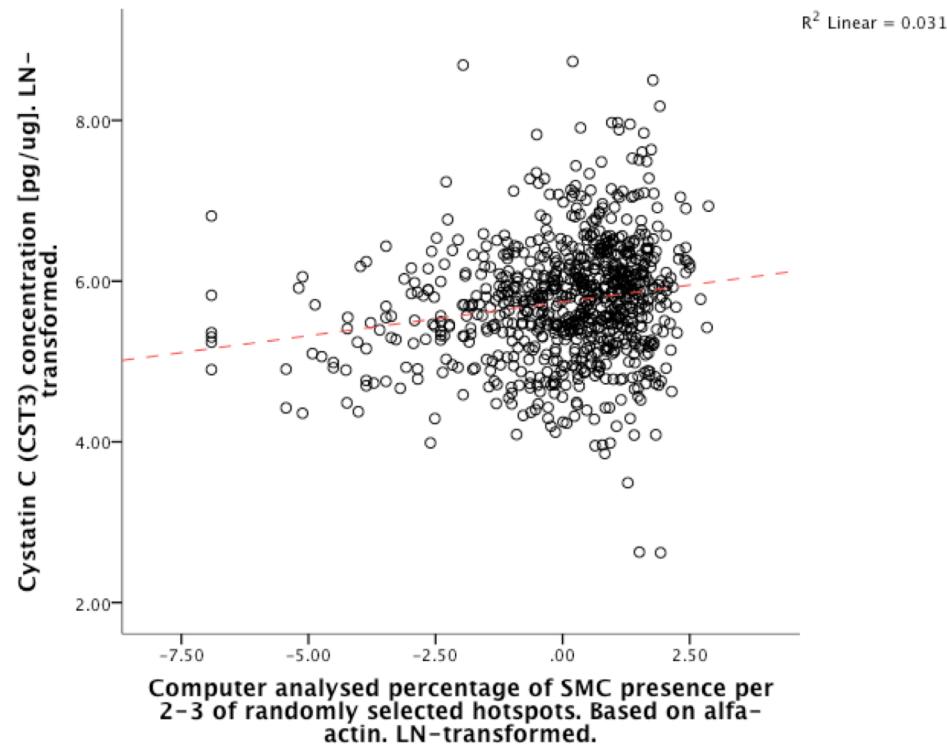
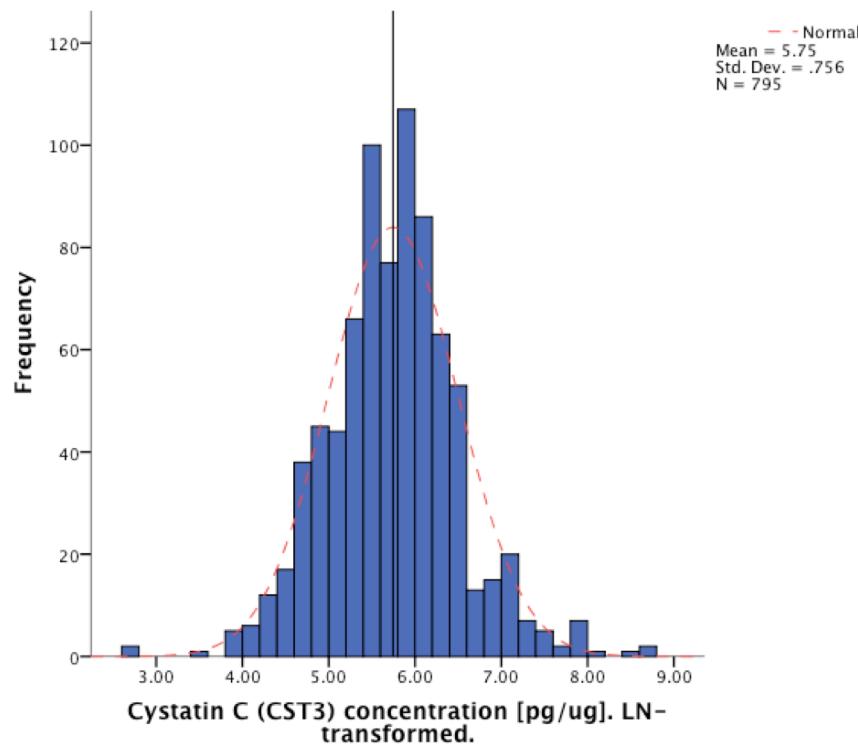
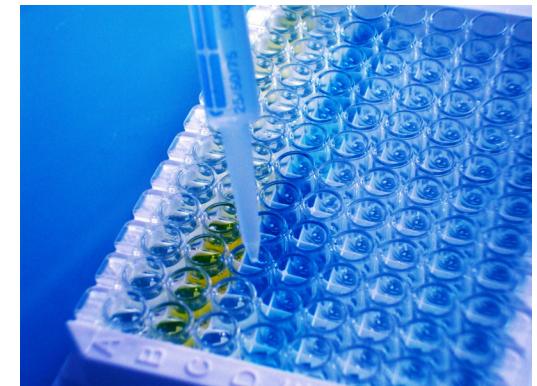
Quantitative Proteomics substantiates Genomic Analyses

- 40 Athero-Express patients selected for individual quantitative plaque proteomics
- Cystatin C protein expression negatively correlated with smooth muscle cells
 - Rho=-0.415, P=0.012
 - Literature: Cystatin C is expressed by SMCs, and markedly decreased in atherosclerotic tissue
- Validation of these results in the remaining Athero-Express CEA cohort:
 - N=1,711 patients
 - Males: 1,174 / Females: 537
 - Average age: 68.03 [years]
 - Males: 67.90 / Females: 68.33
 - Average eGFR: 70.77 [mL/min/1.73m²]
 - Males: 71.95 / Females: 68.04



Luminex Cystatin C plaque levels

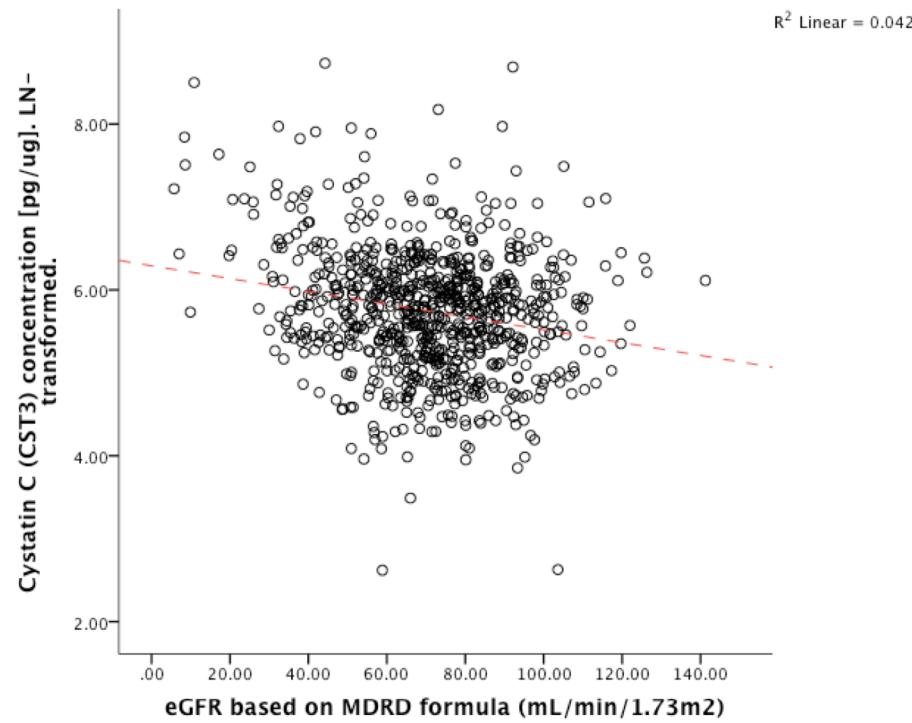
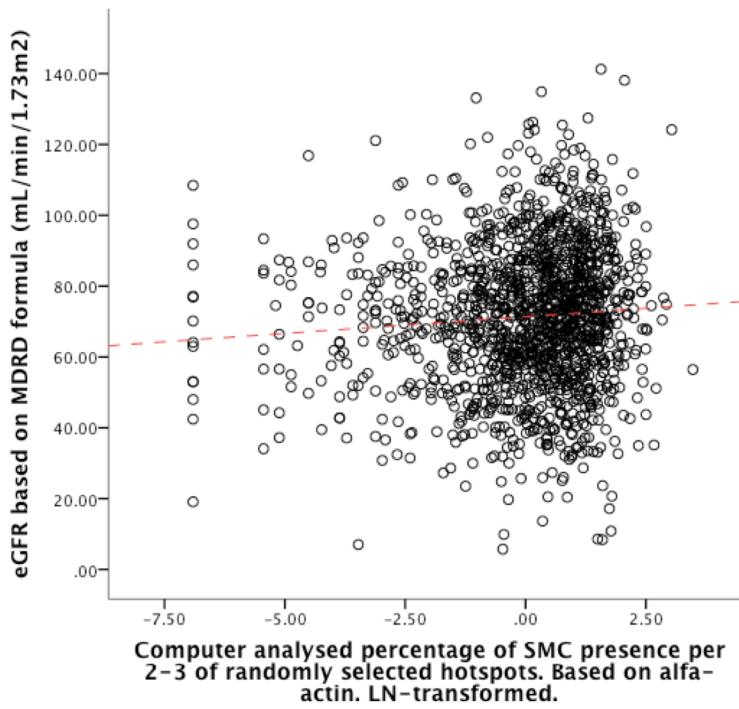
- N = 795
- Rho = 0.185 ($R^2=0.176$), p = 1.10×10^{-7}



Van der Laan *et al.* unpublished

Associations with eGFR

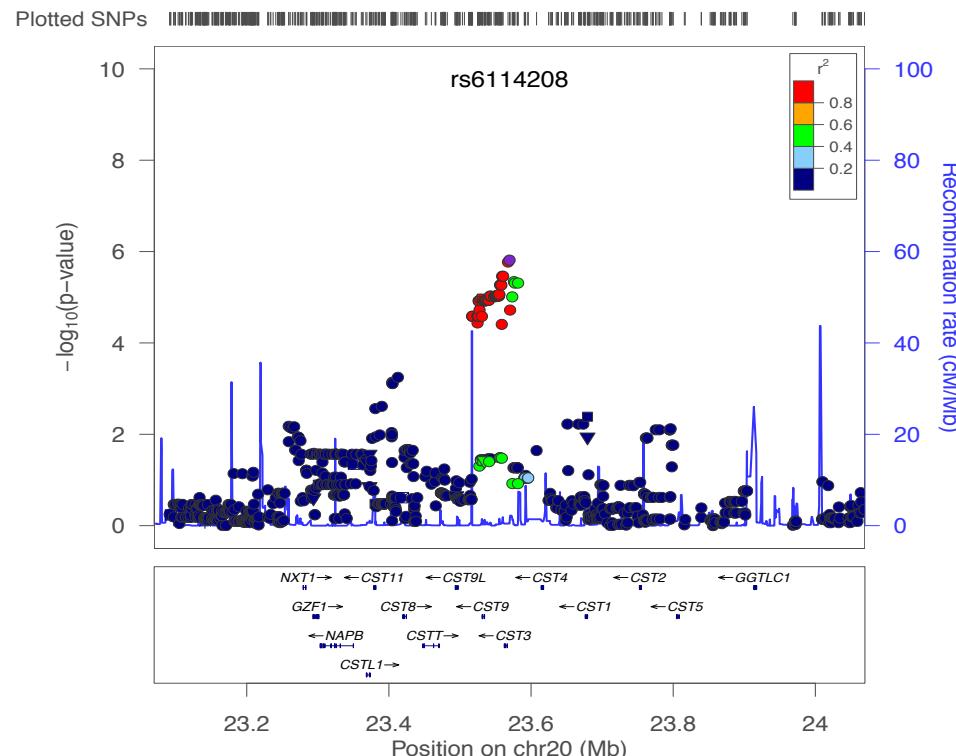
- SMC vs. eGFR: Rho = 0.084 ($R^2 = 0.076$), $p = 1.35 \times 10^{-3}$
- eGFR vs. plaque CST3: Rho = -0.161 ($R^2 = -0.206$), $p = 4.92 \times 10^{-3}$



Van der Laan *et al.* unpublished

CystC-variant associated with SMC in plaques

- In Athero-Express Genomics Study 1 ($N=571$) rs6114208 negatively correlated with SMCs in plaques, $p=1.53\times 10^{-6}$
- When analyzed with additional samples (Athero-Express Genomics Study 2, $N=868$), $p = 0.0083$, $\beta = -0.161$ (0.061), about 10.6% decrease in SMCs



Van der Laan *et al.* unpublished





MENDELIAN RANDOMIZATION

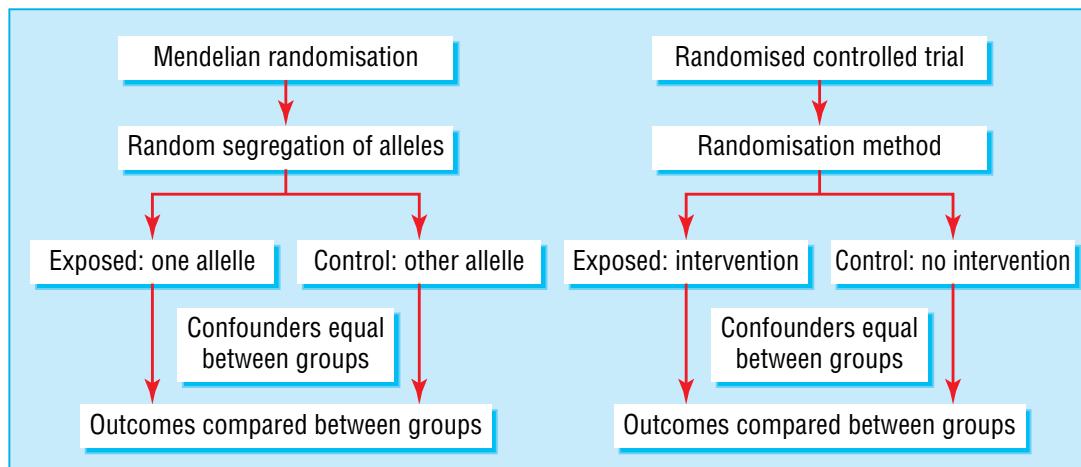
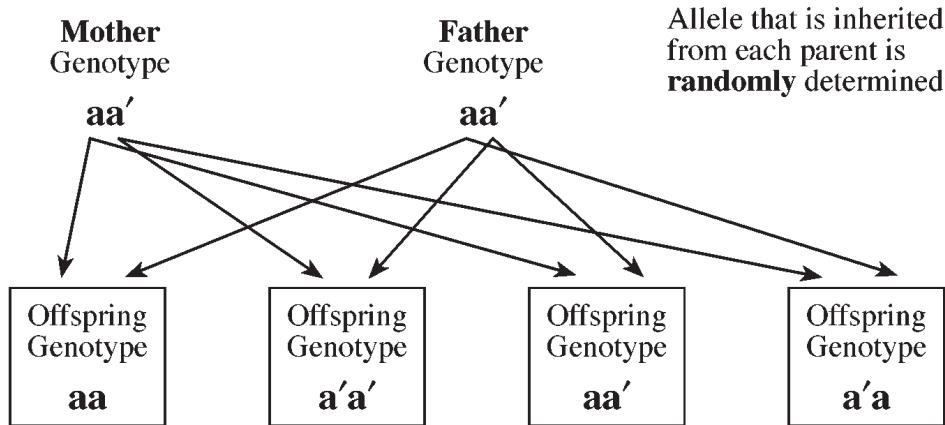


Mendelian randomization

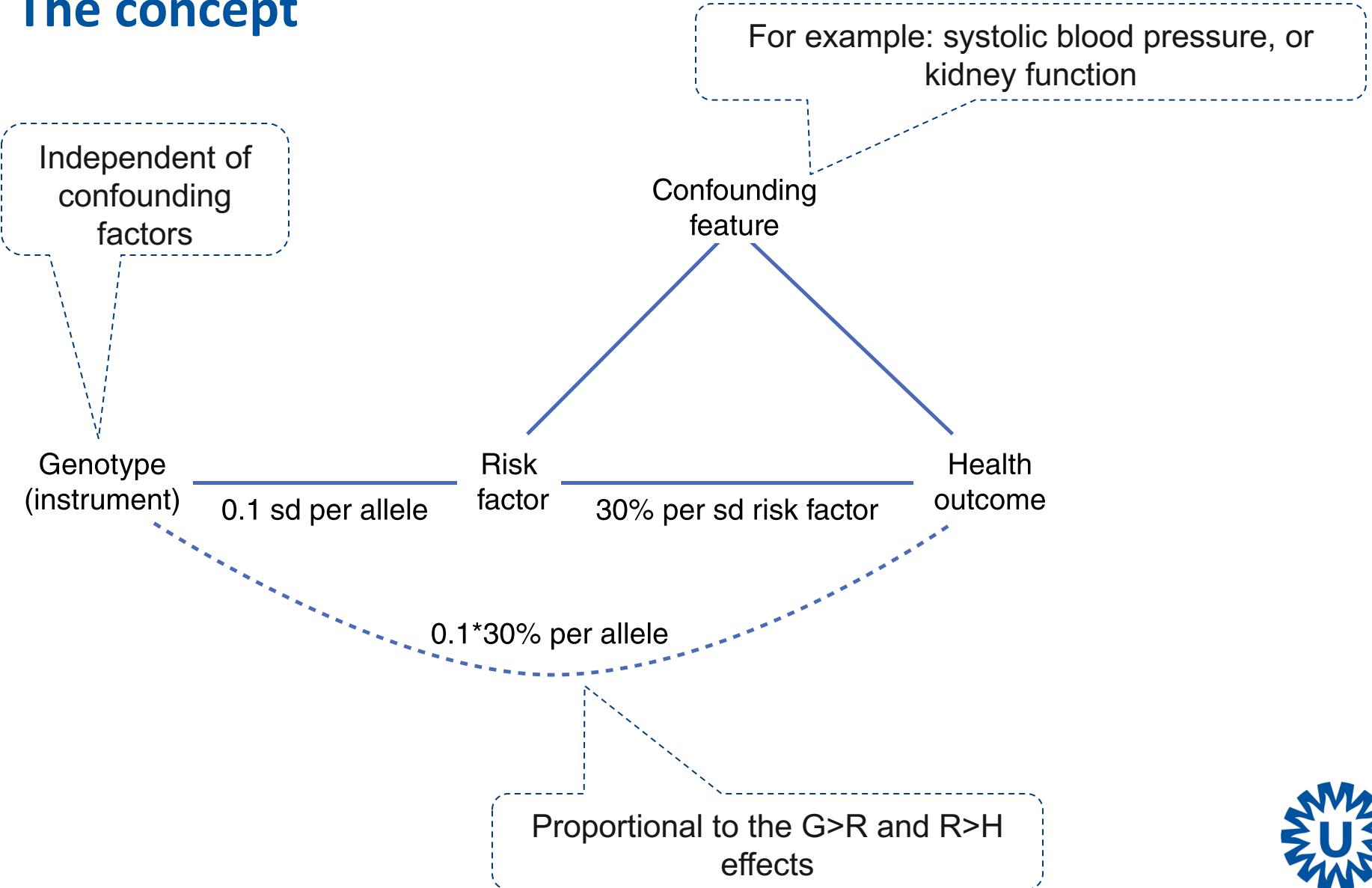
- There is a large body of evidence from animal models, human specimens and epidemiological studies that Cystatin C is associated with CVD
- It appears there is also a correlation between this variant, the amount of CystC, and the number of SMCs in plaques in the Athero-Express Biobank Study (N=1,439)
- Is it causal or is it a bystander?
- Use a genetic variant as a tool to investigate the causal role of Cystatin C in CVD



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



The concept



SNP selection

- One GWAS on Cystatin C, rs1158167 in Framingham Heart Study
- Three (meta-analyses of) GWAS on eGFR_{CystatinC}
 - rs911119, rs13038305
 - eGFR_{CystatinC} = 76.7 x (serum Cystatin C)^{-1.19}
 - Log linear relation between serum Cystatin C 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
<u>rs3827143</u>	6,880	1	20	23,567,617	G	A	0.233	upstream	n/a
<u>rs6114208</u>	8,997	1	20	23,569,734	G	C	0.233	upstream	n/a



Studies included

- There are many more cohorts, but...
- ...we discovered that a similar effort is underway in the US...
- ...but it's focused on the causal relation with CKD
- So ours includes mainly European cohorts
- Maybe we'll be able to collaborate in the future?

Study	<i>N_{total}</i>	<i>N_{SNP}</i>	<i>N_{CystC}</i>	<i>N_{CHD}</i>
<i>3C</i>	6,440	6,345	1,244	1,235
<i>BWHHS</i>	3,413	3,402	-	308
<i>CFS</i>	669	669	262	-
<i>EPIC-NL</i>	6,265	5,192	-	1,430
<i>GOSH</i>	1,478	1,478		105
<i>HRS</i>	5,585	5,585	5,585	-
<i>KORA</i>	4,856	1,867	4,676	341
<i>PIVUS</i>	1,016	949	1,004	175
<i>PREVEND</i>	3,296	3,296	3,296	191
<i>Rotterdam</i>	4,730	5,974	3,906	1,934
<i>SHIP</i>	3,224	3,224	3,212	19
<i>Tromsø</i>	6,175		6,175	1,263
<i>TWINGENE</i>	8,313	6,353	6,768	725
<i>ULSAM</i>	1,221	1,107	1,193	285
<i>WHI</i>	3,074	3,074	3,074	
<i>Whitehall II</i>	4,961	5,011		254
Overall	55,467	50,542	31,146	7,002
<i>CARDIoGRAM</i>	86,959			22,233
<i>METASTROKE</i>	74,393			12,389
<i>CHARGe HF</i>	20,926			2,526

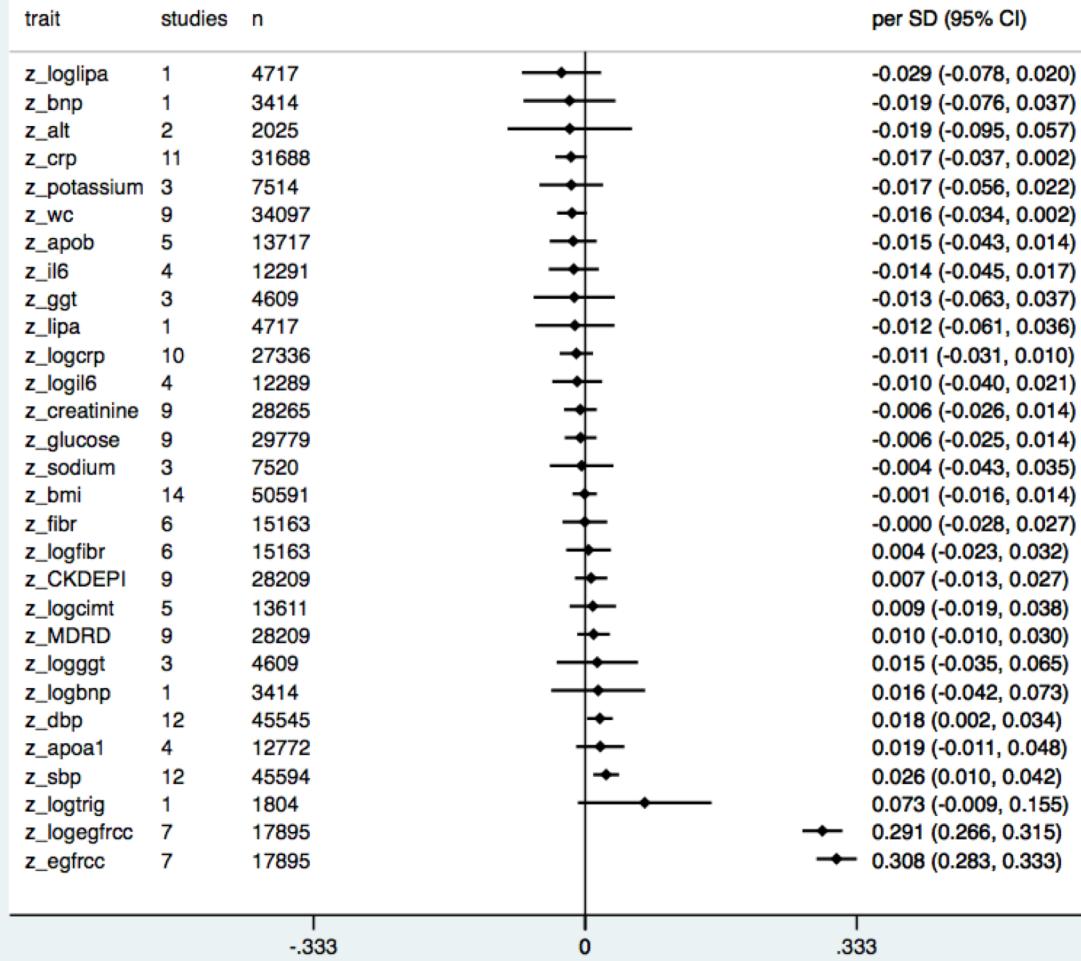
Van der Laan *et al.* unpublished

Cystatin C and risk factors #1

Trait	N	N studies	BETA	P	r^2	P_{het}
Sex	19,589	4	0.093 (0.084-0.101)	5.2×10^{-104}	75.21	0.007
Age	20,782	5	0.011 (0.011-0.012)	$<5.0 \times 10^{-300}$	99.27	6.59×10^{-118}
HDL	20,731	5	-0.154 (-0.163--0.144)	1.21×10^{-207}	35.52	0.184
Systolic BP	20,154	5	0.003 (0.003-0.003)	4.07×10^{-149}	96.12	2.29×10^{-21}
BMI	20,194	5	0.014 (0.013-0.015)	1.6×10^{-158}	88.72	3.71×10^{-7}
Smoking status	20,770	5	0.015 (0.009-0.021)	1.626×10^{-7}	88.56	4.69×10^{-7}
CKDEPI	20,720	5	-0.011 (-0.011--0.011)	$<5.0 \times 10^{-300}$	98.92	1.41×10^{-78}



Cystatin C and risk factors #2



- SBP might be associated with CystC levels
- Strong relation of CystC with eGFR



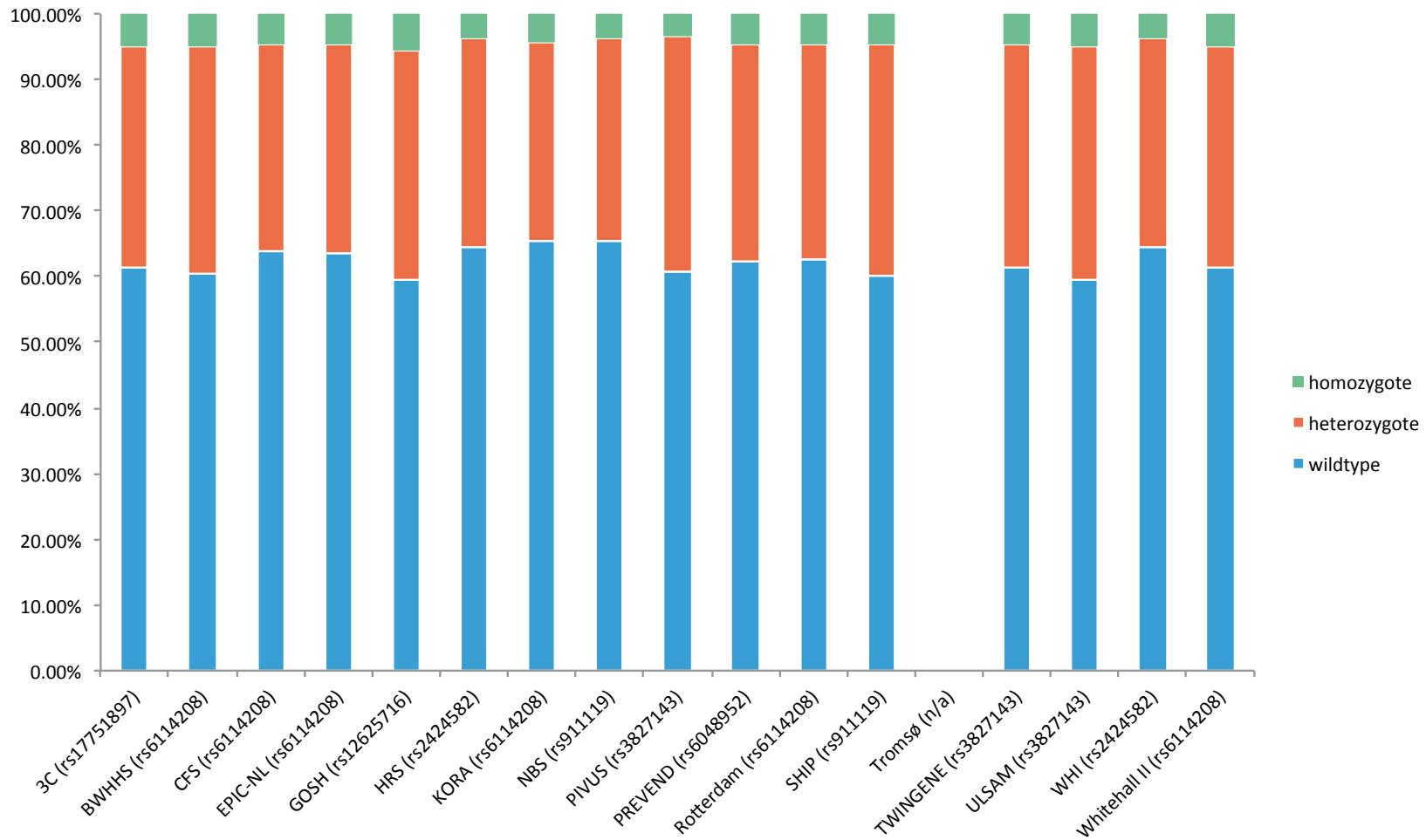
Observational analysis

- “univariate” model: age + sex
- multivariate model: age + sex + HDL + systolic blood pressure + BMI + smoking status + CKDEPI

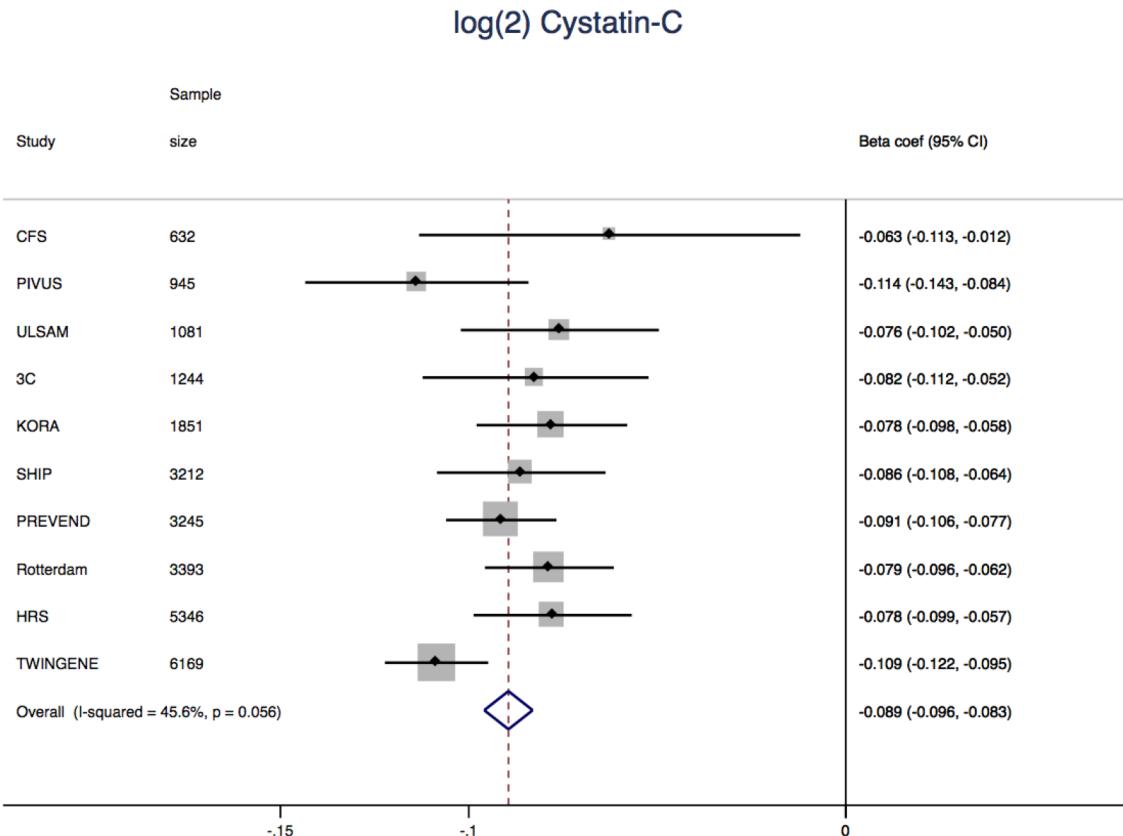
Trait	Model	N cases/controls	N studies	OR (95%CI)	P
IS	univariate	2,149/29,349	8	1.80 (1.33-2.31)	5.90×10^{-5}
	multivariate	2,076/28,056		1.60 (1.05-2.31)	0.028
MI	univariate	1,996/14,833	5	2.10 (1.41-3.11)	2.30×10^{-4}
	multivariate	1,970/14,652		1.30 (0.89-1.86)	0.174
HF	univariate	1,243/16,623	5	3.90 (2.50-6.12)	2.13×10^{-9}
	multivariate	1,159/15,470		3.90 (2.20-7.08)	4.31×10^{-6}
Fatal CVD	univariate	761/8,178	3	2.00 (1.49-2.81)	9.79×10^{-6}
	multivariate	736/8,100		2.30 (1.13-4.59)	0.021
CHD	univariate	3,115/22,710	7	2.20 (1.72-2.73)	7.86×10^{-11}
	multivariate	2,980/21,476		1.60 (1.15-2.20)	5.19×10^{-3}



Are frequencies comparable?



SNP vs. Cystatin C

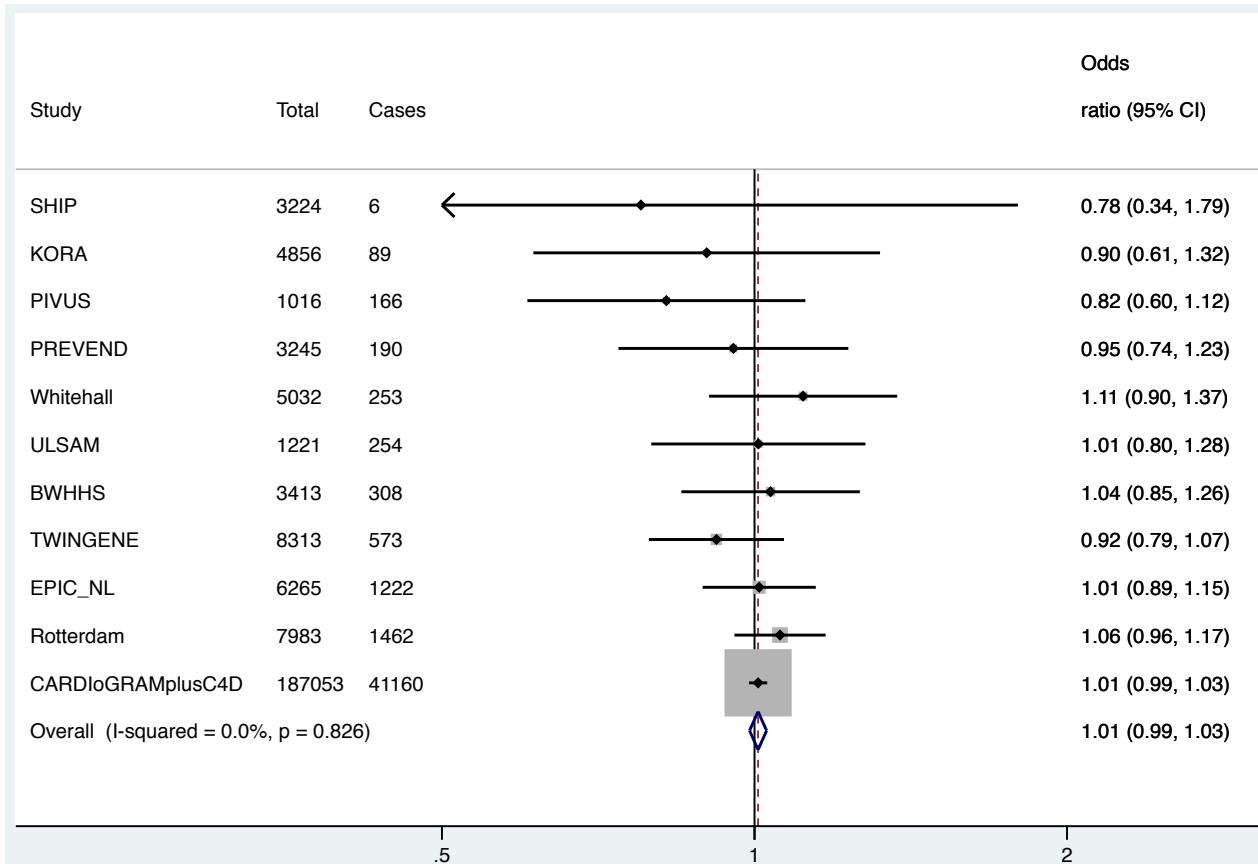


- Per minor allele there is $\approx 6\%$ decrease in CystC [mg/L]
- $\beta = -0.089 [-0.096-0.083]$, $p = 2.10 \times 10^{-164}$, $N = 24,642$
- This explains $\approx 2.5\%$ of the phenotypic variation

Van der Laan *et al.* unpublished



SNP vs. CVD



- The SNP affecting CystC levels is not associated with CHD
- OR = 1.01 [0.99-1.03], p = 0.41, N>49,165 cases

Van der Laan *et al.* unpublished



Conclusions

- Cystatin C downregulated in atherosclerotic plaques
- There is a correlation between the number of SMCs, eGFR and plaque-CystC
- Variants in CystC are associated with SMCs in plaques
- Strong epidemiological evidence for a role of CystC in CVD
- Variants in CystC associated with serum CystC levels
- Variants in CystC are not associated with CVD



HDL protective of MI?

- Various variants known to influence HDL levels
- HDL levels are associated with lower risk of MI (or CAD)
- Some also associated with the risk of MI (or CAD)
- Is a lower HDL level causal to a lower risk for MI?



Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study

Benjamin F Voight*, Gina M Peloso*, Marju Orho-Melander, Ruth Fririke-Schmidt, Maja Borbalic, Majken Kjeldsen, George H Cindy, Hilma H ölm, Eric L Ding, Toby Johnson, Heribert Schunkert, Nilesh J Samani, Robert Clarke, Jemma C Hopewell, John F Thompson, Mingyao Li, Gudmar Thorleifsson, Christopher Newton-Cheh, Kiran Musunuru, James P Pirruccello, Danish Saleheen, Li Chen, Alexandre F Stewart, Arne Schillert, Unnur Thorsteinsdóttir, Guðmundur Þorgerðsson, Sónia Arand, James C Engert, Thomas Morgan, John Spertus, Monika Stoll, Klaus Berger, Nicola Martinielli, Domenico Girelli, Pascal P McKeown, Christopher C Patterson, Stephen E Epstein, Joseph Devaney, Mary-Susan Burnett, Vincent Mooser, Samuli Ripatti, Ida Surakka, Markku S Nieminen, Juha Sirola, Marja-Liisa Lokki, Markus Perola, Aki Havulinna, Ulf de Faire, Bruna Gigante, Erik Ingelsson, Tanja Zeller, Philipp Wild, Paul I IV de Bakker, Olaf H Klüngel, Anke-Hilse Maitland-van der Zee, Bas J M Peters, Anthoniou de Boer, Diederick E Grobbee, Pieter W Kamphuisen, Vera H M Deneer, Clara C Elbers, N Charlotte Onland-Moret, Marten H Hofker, Cisca Wijmenga, WM Monique Verschuren, Jolanda M A Boer, Yvonne T van der Schouw, Asif Rasheed, Philippe Frossard, Serkalem Demissie, Cristen Willer, Ron Do, Jose M Ordovas, Gonçalo R Abecasis, Michael Boehnke, Karen L Mihalek, Mark J Daly, Candace Guiducci, Noël P Butt, Aarti Surti, Elena Gonzalez, Shaun Purcell, Stacey Gabriel, Jaume Marrugat, John Peden, Jeanette Erdmann, Patrick Diemert, Christina Willenborg, Inke R König, Marcus Fischer, Christian Hengstenberg, Andreas Ziegler, Ian Buyssechaert, Diether Lambrechts, Frans Van de Werf, Keith A Fox, Nour Eddine El Mokhtar, Diana Rubin, Jürgen Schrezenmeier, Stefan Schreiber, Anne Schäfer, John Danesh, Stefan Blanckenberg, Robert Roberts, Ruth McPherson, Hugh Watkins, Alastair S Hall, Kim Overvad, Eric Rimm, Eric Boerwinkle, Anne Tybjærg-Hansen, L Adrienne Cupples, Muredach P Reilly, Olle Melander, Pier M Mannucci, Diego Ardissino, David Siscovick, Roberto Eliasua, Kari Stefansson, Christopher J O'Donnell, Veikko Salomaa, Daniel J Rader, Leena Peltonen, Stephen M Schwartz, David Altschuler, Sekar Kathiresan

Summary

Lancet 2012; 380: 572-80
Published Online
May 17, 2012
[http://dx.doi.org/10.1016/S0140-6736\(12\)60312-2](http://dx.doi.org/10.1016/S0140-6736(12)60312-2)

This online publication has been corrected. The corrected version first appeared at thelancet.com on June 1, 2012

See Comment page 543

*These authors contributed equally to this work

Affiliations listed at end of paper

Correspondence to:
Dr Sekar Kathiresan, Center for Human Genetic Research and Cardiovascular Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA
skathiresan@partners.org

Findings Carriers of the *LIPG* 396Ser allele (2·6% frequency) had higher HDL cholesterol (0·14 mmol/L higher, $p=8\times 10^{-13}$) but similar levels of other lipid and non-lipid risk factors for myocardial infarction compared with non-carriers. This difference in HDL cholesterol is expected to decrease risk of myocardial infarction by 13% (odds ratio [OR] 0·87, 95% CI 0·84–0·91). However, we noted that the 396Ser allele was not associated with risk of myocardial infarction (OR 0·99, 95% CI 0·88–1·11, $p=0·85$). From observational epidemiology, an increase of 1 SD in HDL cholesterol was associated with reduced risk of myocardial infarction (OR 0·62, 95% CI 0·58–0·66). However, a 1 SD increase in HDL cholesterol due to genetic score was not associated with risk of myocardial infarction (OR 0·93, 95% CI 0·68–1·26, $p=0·63$). For LDL cholesterol, the estimate from observational epidemiology (a 1 SD increase in LDL cholesterol associated with OR 1·54, 95% CI 1·45–1·63) was concordant with that from genetic score (OR 2·13, 95% CI 1·69–2·69, $p=2\times 10^{-10}$).

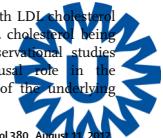
Interpretation Some genetic mechanisms that raise plasma HDL cholesterol do not seem to lower risk of myocardial infarction. These data challenge the concept that raising of plasma HDL cholesterol will uniformly translate into reductions in risk of myocardial infarction.

Funding US National Institutes of Health, The Wellcome Trust, European Union, British Heart Foundation, and the German Federal Ministry of Education and Research.

Introduction

Cholesterol fractions such as LDL and HDL cholesterol are among the most commonly measured biomarkers in clinical medicine.¹ Observational studies have shown that LDL and HDL cholesterol have opposing associations

with risk of myocardial infarction, with LDL cholesterol being positively associated and HDL cholesterol being inversely associated.^{2,3} However, observational studies cannot distinguish between a causal role in the pathological process and a marker of the underlying



The answer in three steps... #1

- Various variants associated with HDL levels

	Chromosome	Gene(s) of interest within or near associated interval	Major allele, minor allele (minor allele frequency)*	Modelled allele	Effect of modelled allele on plasma HDL cholesterol (mmol/L)*	Effect of modelled allele on plasma triglycerides (mmol/L)*	Effect of modelled allele on plasma LDL cholesterol (mmol/L)*	Sample size (MI cases/MI-free controls)	For modelled allele, observed change in MI risk (%; 95% CI)	For modelled allele, p value for association with MI
rs17482753	8p21	LPL†	G, T (0.10)	T	0.08	-0.24	..	19 139/50 812	-12% (-16 to -7)	4×10 ⁻⁷ †
rs17321515	8q24	TRIB1†	A, G (0.45)	G	0.02	-0.11	-0.05	19 139/50 812	-7% (-9 to -4)	2×10 ⁻⁶ †
rs6589566	11q23	APOA1-APOC3-APOA4-APOA5†	A, G (0.07)	A	0.05	-0.27	-0.09	18 310/49 897	-10% (-15 to -5)	8×10 ⁻⁵ †
rs4846914	1q42	GALNT2†	A, G (0.40)	A	0.02	-0.03	..	19 139/50 812	-3% (-6 to -1)	0.02†
rs2967605	19p13	ANGPTL4†	C, T (0.16)	C	0.05	-0.07	..	13 595/16 423	-5% (-10 to -1)	0.03†
rs3764261	16q13	CETP†	C, A (0.32)	A	0.10	..	-0.03	16 503/46 576	-4% (-7 to 0)	0.04†
rs61755018 (Asn396Ser)	18q21	LIPG	A, G (0.015)	G	0.14‡	17 165/49 077	-6% (-18 to 9)	0.41
rs17145738	7q11	MLXIPL	C, T (0.11)	T	0.03	-0.15	..	19 139/50 812	-1% (-4 to 3)	0.61
rs3890182	9q31	ABCA1	G, A (0.14)	G	0.03	..	0.05	19 139/50 812	-1% (-5 to 4)	0.76
rs2338104	12q24	MMAB, MVK	G, C (0.46)	G	0.03	19 139/50 812	0% (-3 to 3)	0.85
rs471364	9p22	TTC39B	T, C (0.12)	T	0.03	15 693/47 098	0% (-5 to 5)	0.97
rs2271293	16q22	LCAT	G, A (0.11)	A	0.03	19 139/50 812	4% (-1 to 8)	0.10
rs174547	11q12	FADS1-FADS2-FADS3	T, C (0.33)	T	0.03	-0.06	..	19 139/50 812	3% (-1 to 6)	0.11
rs1800588	15q22	LIPC	C, T (0.22)	T	0.05	0.07	..	17 917/49 514	4% (0 to 7)	0.04
rs16988929	20q13	HNF4A	C, T (0.01)	T	0.01	17 041/20 137	31% (12 to 54)	9×10 ⁻⁴

*Data presented from a meta-analysis of seven cohorts (n up to 19 840) as presented in reference 16; the effect of each SNP on a lipid trait was modelled if the association of the SNP with a plasma lipid trait exceeded nominal significance ($p<0.05$). †Loci and SNPs that exceeded nominal significance ($p<0.05$) for association of modelled allele with MI; all modelled alleles increased HDL cholesterol. ‡Effect size presented is from the Atherosclerosis Risk in Communities Study.

Table 2: Association of myocardial infarction (MI) with single nucleotide polymorphisms (SNPs) previously found to relate to plasma HDL cholesterol

The answer in three steps... #2

- *LIPG* variant associates with HDL levels

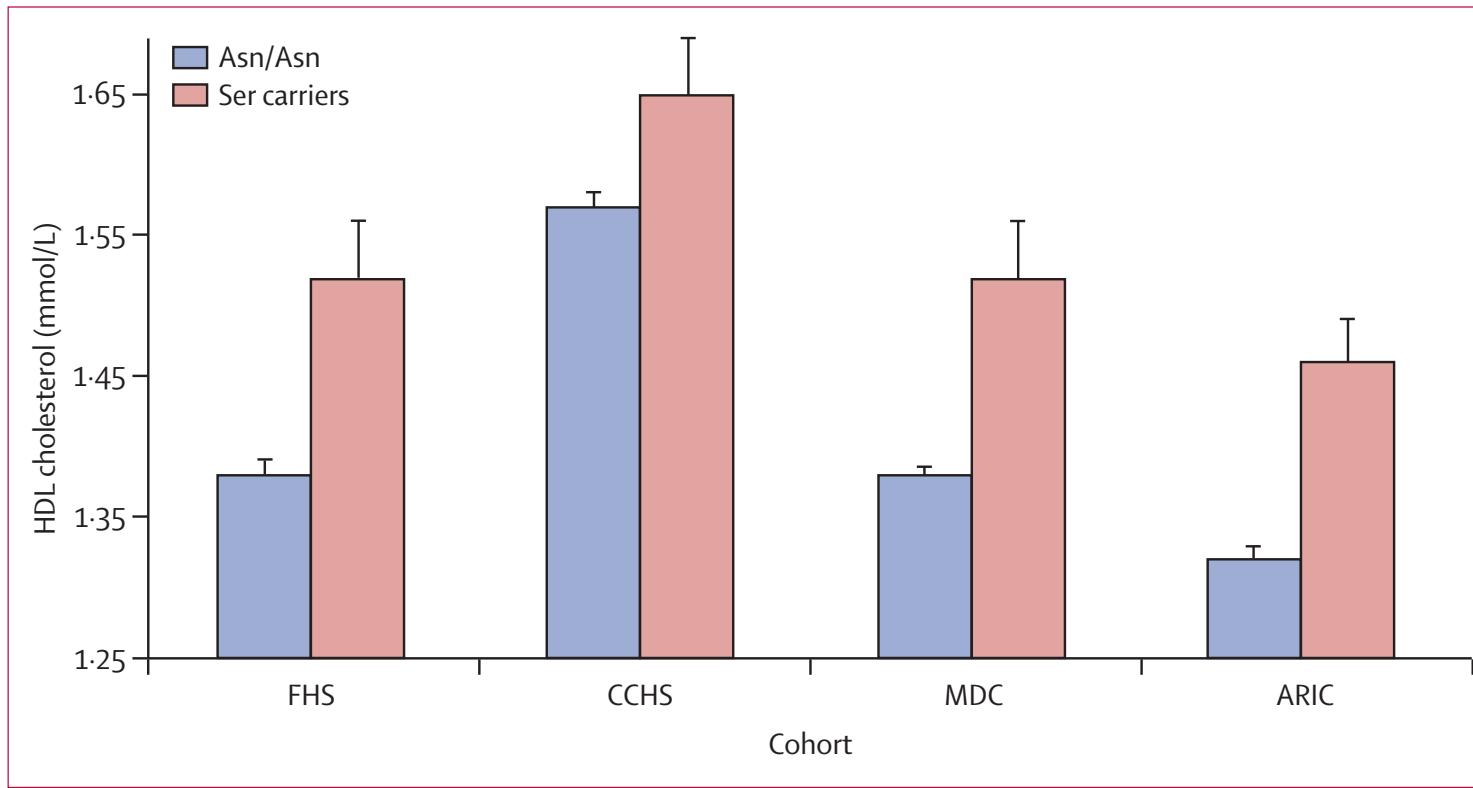


Figure 1: Plasma HDL cholesterol concentrations in carriers versus non-carriers of the Ser allele at the *LIPG* Asn396Ser polymorphism

Error bars show standard error. FHS=Framingham Heart Study. CCHS=Copenhagen City Heart Study. MDC=Malmo Diet and Cancer Study. ARIC=Atherosclerosis Risk in Communities Study.



The answer in three steps... #3

- No association with MI...

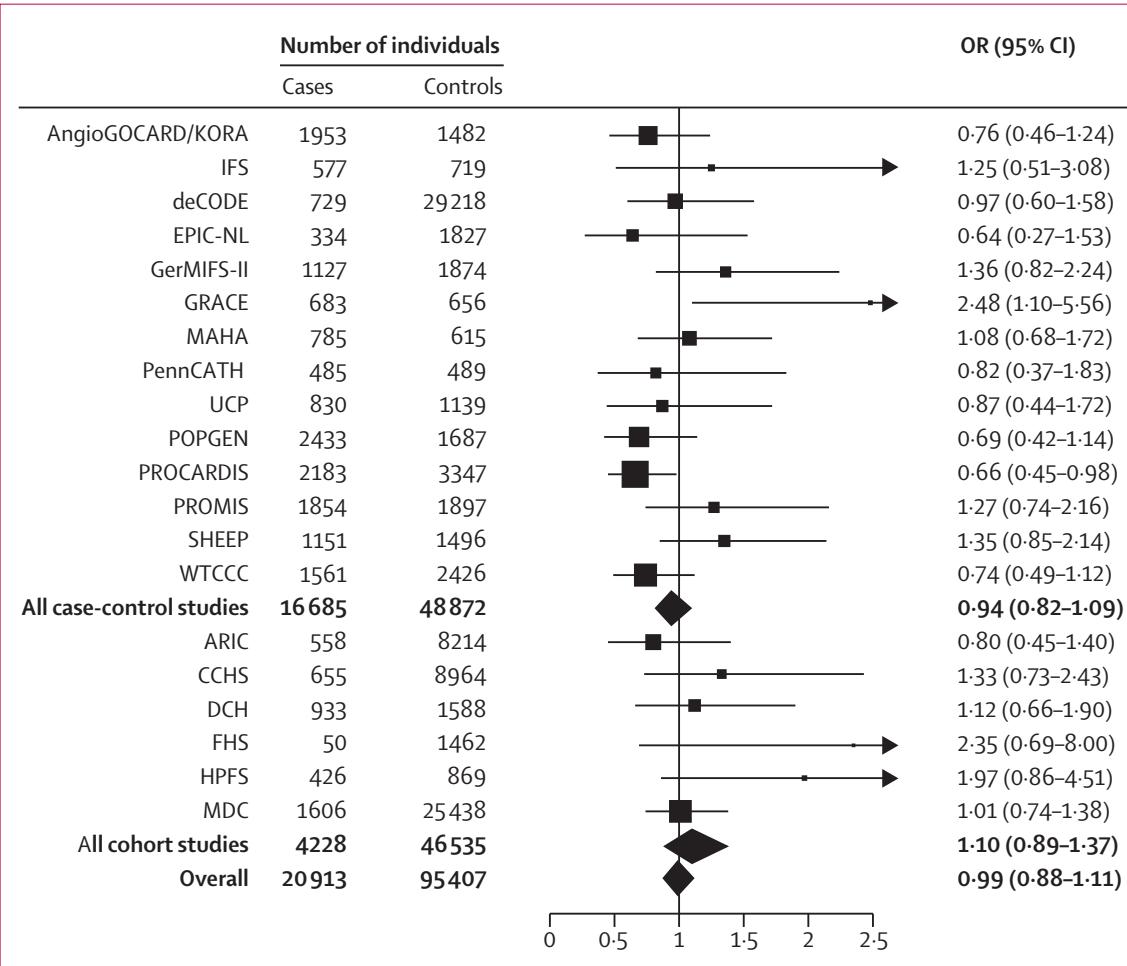


Figure 2: Association of LIPG Asn396Ser with myocardial infarction in 116 320 participants from 20 studies
In each study, the HDL-cholesterol-raising serine allele was modelled.



Cardiovascular Genetic Research

Experimental Cardiology Laboratory

Prof. Dr. G. Pasterkamp

Dr. Hester M. den Ruijter

Dr. Jessica van Setten

Medical Genetics

Prof. Dr. P.I.W. de Bakker

Drs. Sara L. Pulit

Cardiology

Dr. F.W. Asselbergs

Magdalena Harakalova

Research topics

Biomarker Discovery & Validation

Athero-Express / CTMM: Circulating Cells

Sex differences in Cardiovascular Disease

Athero-Express / CTMM / UCORBIO

Ischemic stroke

GWAS

Cardiovascular Genomics

Next-Generation Sequencing | Pharmacogenomics

S.W. van der Laan – s.w.vanderlaan-2@umcutrecht.nl



UMC Utrecht



Genetic Investigation of
ANthropometric Traits

