

Cardiovascular Genomics

An introduction to the analysis of genetic variants
in cardiovascular disease

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What we'll discuss today...

- Recapture Some Basic Genetics (again)
- Genetics of Advanced Atherosclerotic Disease
- Genetic Burden for Disease (Risk)
- Genetics, Biomarkers & Disease



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Recapturing Some Basic Genetics (again)

THE HUMAN GENOME



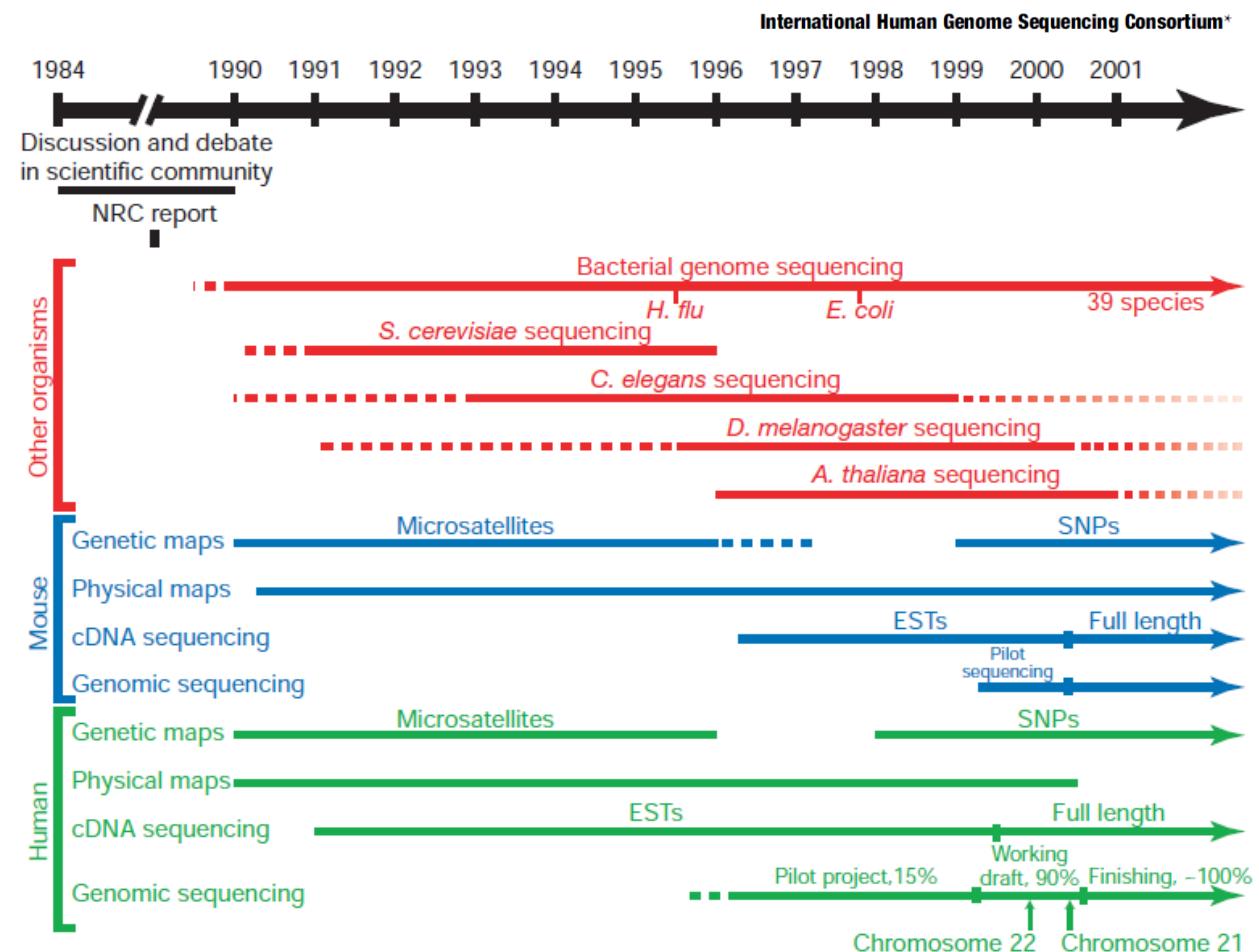
What did you remember?

- SNP?
- Other genetic variations?
- Manhattan?
- LD?
- Affymetrix? Illumina?
- HapMap?
- Common Disease/Common Variant?
- Study designs? Types of diseases?
- Amino acid?
- Nucleic acid?

Human Genome Project: 2001

articles

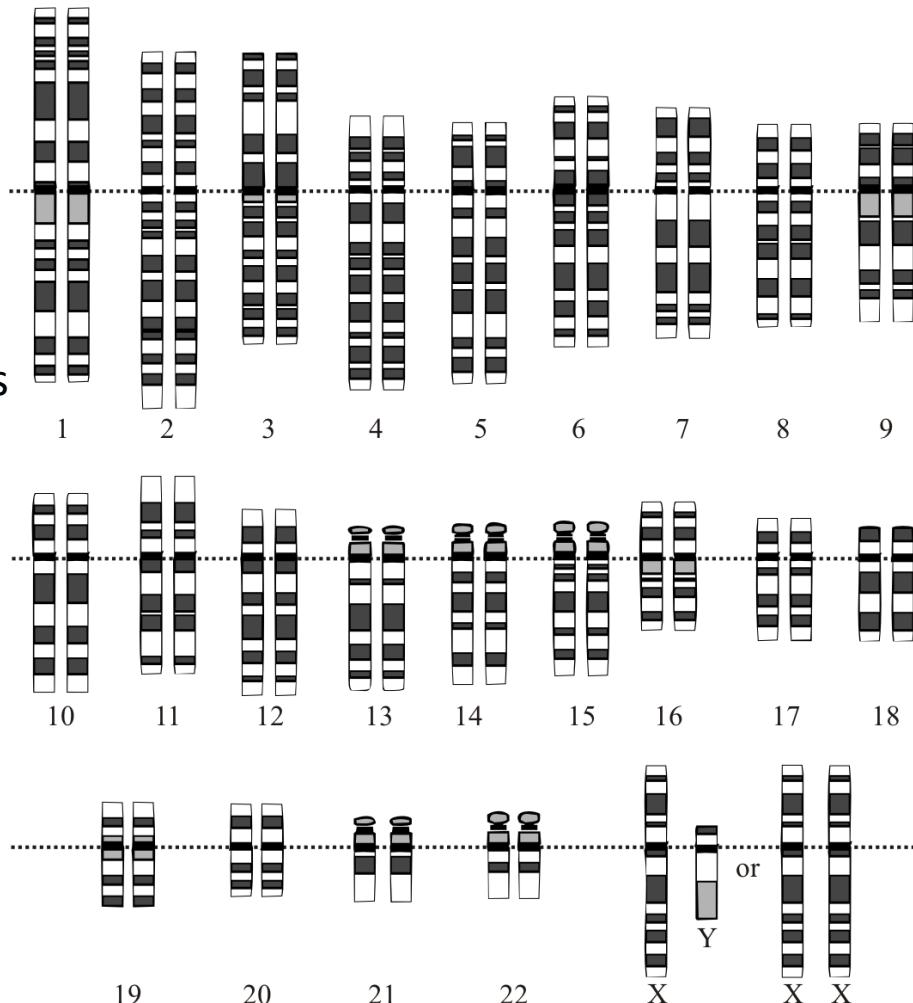
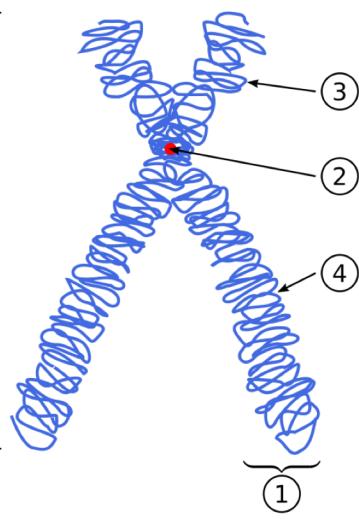
Initial sequencing and analysis of the human genome



- Largest publication ever in *Nature*, February 2001: 62 pages
- “Back-to-back with a publication by Craig Venter, *et al.* in *Science*

Human Genome: *the chromosomes*

- Chromosomes:
 - Autosomes, 22 pairs
 - Sex-chromosomes, X and Y
 - Mitochondrial chromosome
 - One copy of one (part) of the chromosome(s) of each of your parents
 - 1) chromatid
 - 2) centromere
 - 3) short arm, p
 - 4) long arm, q



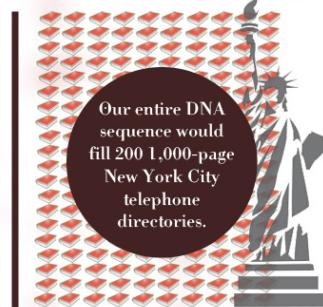
Human Genome: *some statistics*

- 3.2 billion base pairs in the haploid genome
- \approx 20,000-25,000 genes
 - \approx 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
 - Variable number of tandem repeats (VNTR)
 - Copy-number variations (CNV)
 - Transposable elements
 - Viral or bacterial origins
- (So there's no such thing as "junk DNA"...)

Our entire DNA sequence is called a genome... and there's an estimated **3,000,000,000** DNA bases in our genome.

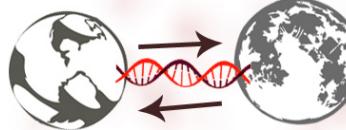


A complete 3 billion base genome would take **3 GIGABYTES OF STORAGE SPACE.**



99.9% OF OUR DNA SEQUENCE IS THE SAME AS OTHER HUMANS'.

IF YOU UNWRAP ALL OF THE DNA YOU HAVE IN ALL YOUR CELLS, YOU COULD REACH THE MOON **6000 TIMES.**



99%

This **0.1% DNA DIFFERENCE** between us may have to do with the number of nucleotides in a person's DNA.

When DNA is copied in to a new life, the nucleotides are either gained or lost in the process. This gain or loss results in our differences.



= **50 YEARS**

It would take a person typing 60 words per minute, 8 hours a day, around 50 years to type the human genome.

ATGCCGATCGTACGACACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCATCGTACTGCATCGATCCATT
TACTGACTGCATCGTACTGACTGCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCCACATATCGTTACCCAT
CATCGTACTGACTGTCTAGTCTAAACACATCCCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCCAGCATCCA
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GCCGATCGTACGACACATATCGTCATCGTACTGCCCTACGGGACTGTCTAGTCTAAACACATCCATCGTACTGACTGCATCG
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CTGCTAGTCTAAACACATCCATCGTACTGACTGCATCGTACGCCGATCGTACGACACATATCGTCATCGTACTGCCCTACGG
CTGCTAGTCTAAACACATCCATCGTACTGACTGCATCGTACTGACTGCATCGTACTGACTGCACATATCGTCATACATAGAC
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GTACTGACTGTCTAGTCTAAACACATCCCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCACTTACCCATG
ATCGTACTCGTACTGACTGTCTAGTCTAAACACATCCCACACTGTCTAGTCTAAACACATCCATCGTACTGACTGCATCGTAC
CGATCGTACGACACATATCGTCATCGTACTGCCCTACGGGACTGTCTAGTCTAAACACATCCATCGTACTGACTGCATCGTAC

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 99.9% similar between individuals
- 0.1% different → individual point variations
- Single-nucleotide polymorphisms

articles

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

The International SNP Map Working Group*

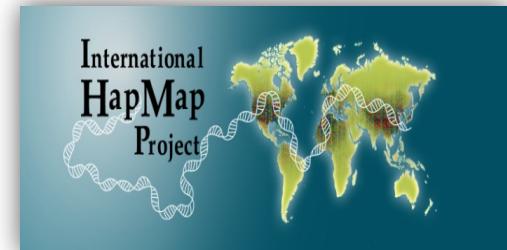
*A full list of authors appears at the end of this paper.

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.

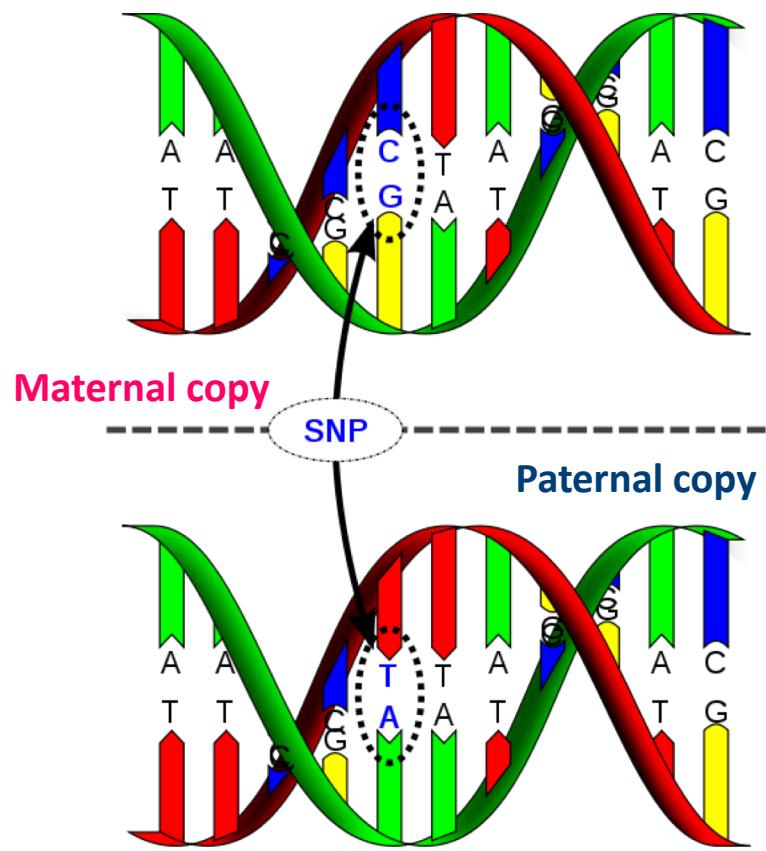
Single-Nucleotide Polymorphism

a genetic variation as proxy

- Single-nucleotide polymorphism (SNP)
- “one base pair variation”
 - > 1% general population (common)
 - ≈10 million SNPs ($\approx 0.25\%$ genome)
 - Makes you and me unique
- SNPs are common variants which are used as proxies of the actual genomic variation

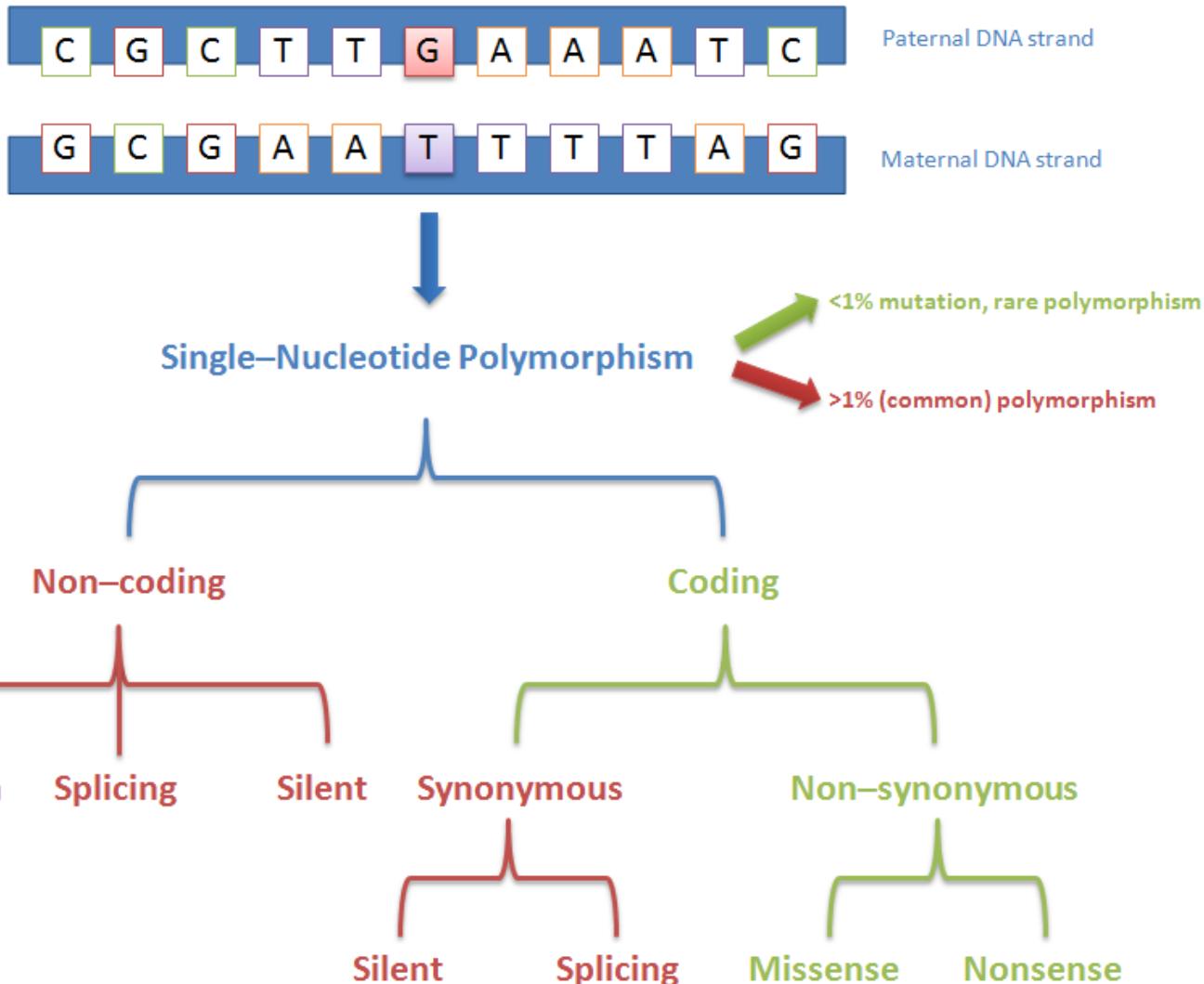


www.hapmap.org

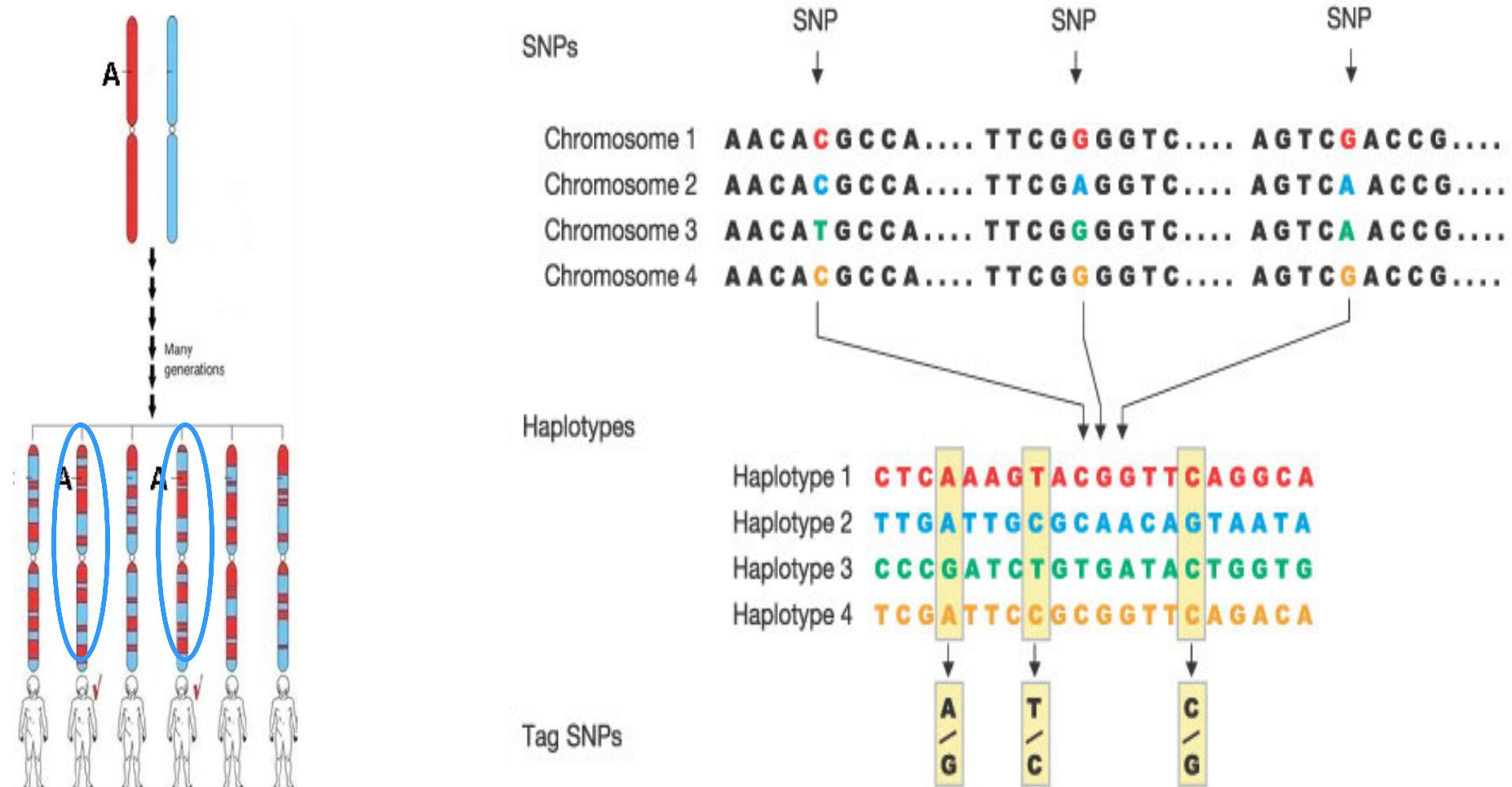


Types of SNPs

Chromosome



SNPs → Haplotype → tagSNP

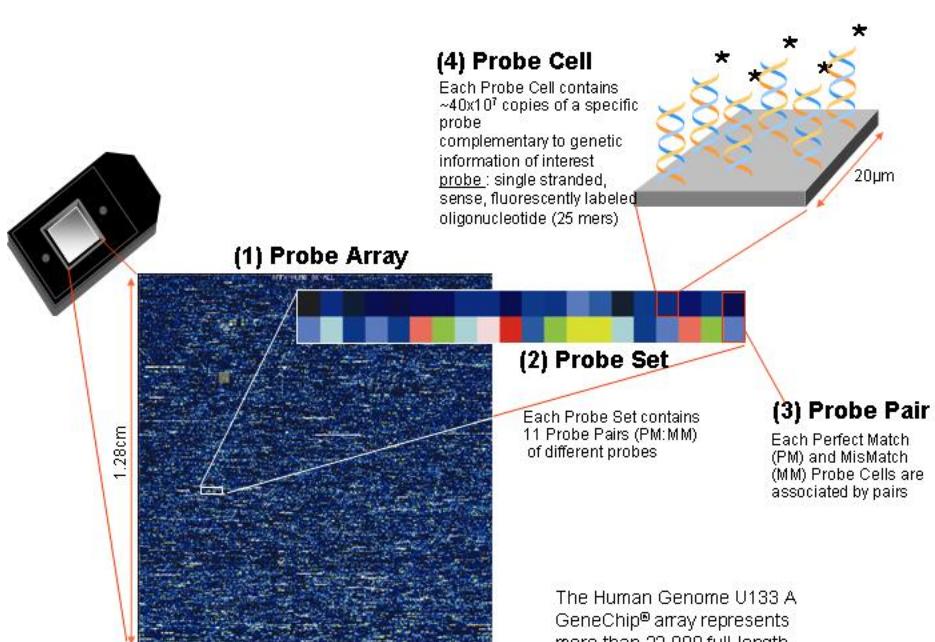
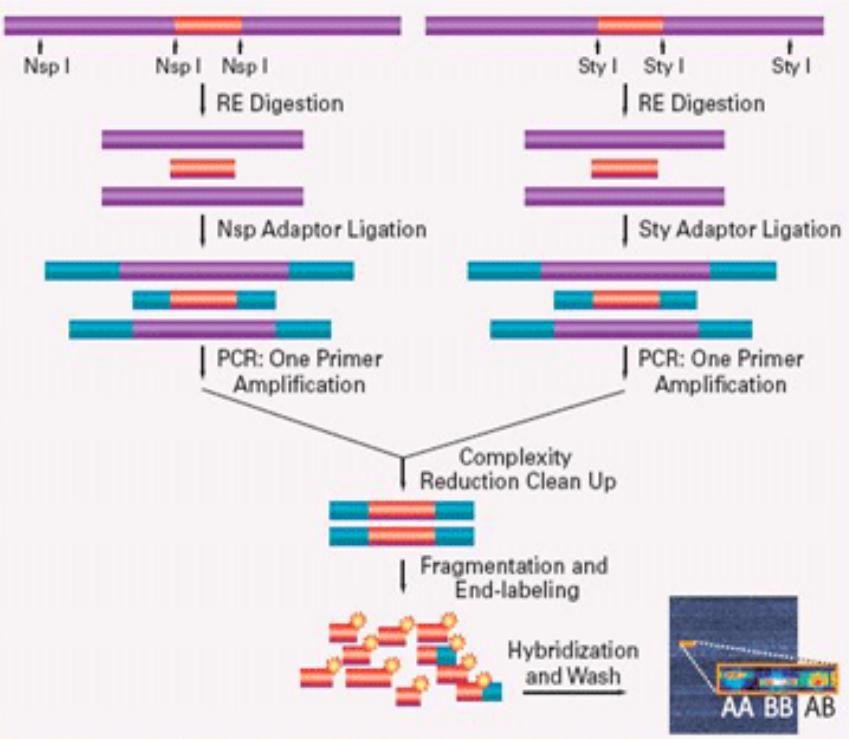


Genotyping Platforms



SNP “genotyping”

The fifth-generation Whole-genome Sampling Assay.



Genotyping Platforms: *examples*

- Affymetrix Genome-Wide Human SNP Array 5.0
 - 500,568 SNPs
 - Chromosomal coverage
 - Good representation of autosomal chromosomes
 - X-chromosome poor representation
 - No Y-chromosome representation
 - No mitochondrial chromosome representation
- Illumina Human660W-Quad v1
 - 657,366 SNPs
 - Chromosomal coverage
 - Good representation of autosomal chromosomes
 - X-chromosome reasonable representation
 - Y-chromosome poor representation
 - Mitochondrial chromosome poor representation

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

What type of disease are we looking at?

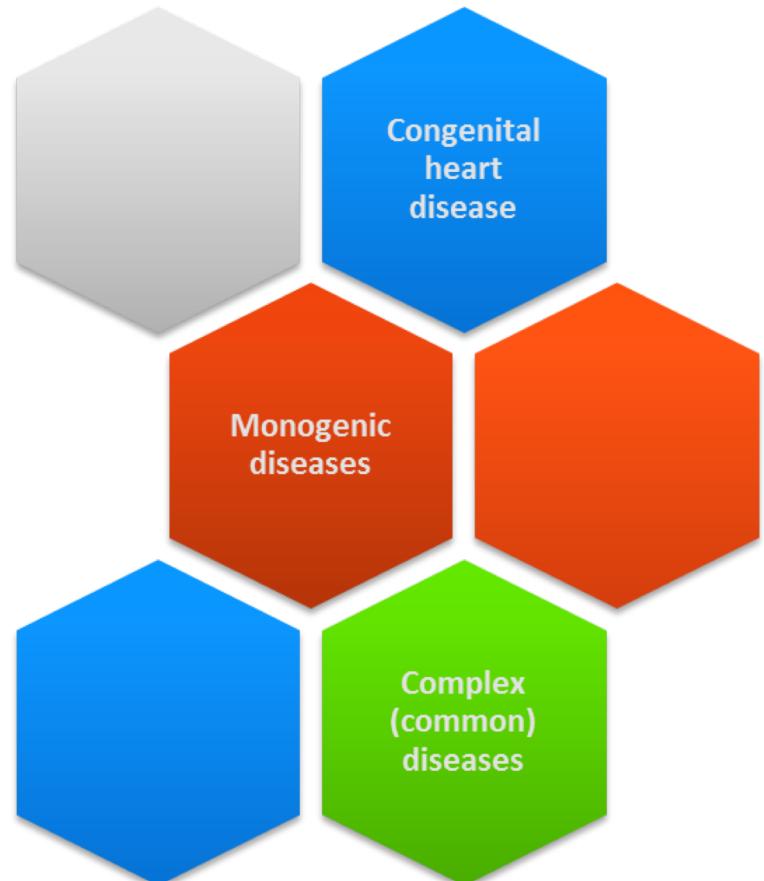
monogenic diseases

- **Congenital heart disease**

- Atrial septal defects
- Ventricular septal defects
- Electrical septal defects

- **Monogenic diseases**

- Mendelian pattern
 - Autosomal dominant, e.g.:
 - Marfan Syndrome
 - Familial hypercholesterolemia
 - Autosomal recessive , e.g.:
 - Sickle cell anemia
 - Cystic fibrosis
 - X-linked , e.g.:
 - Duchene muscular dystrophy
 - Y-linked/Mitochondrial



What type of disease are we looking at?

complex diseases

- Complex diseases

 - Polygenic, multifactorial diseases

 - Diabetes mellitus
 - Asthma
 - Cardiovascular disease
 - Hypertension

Each gene contributes a little to the disease

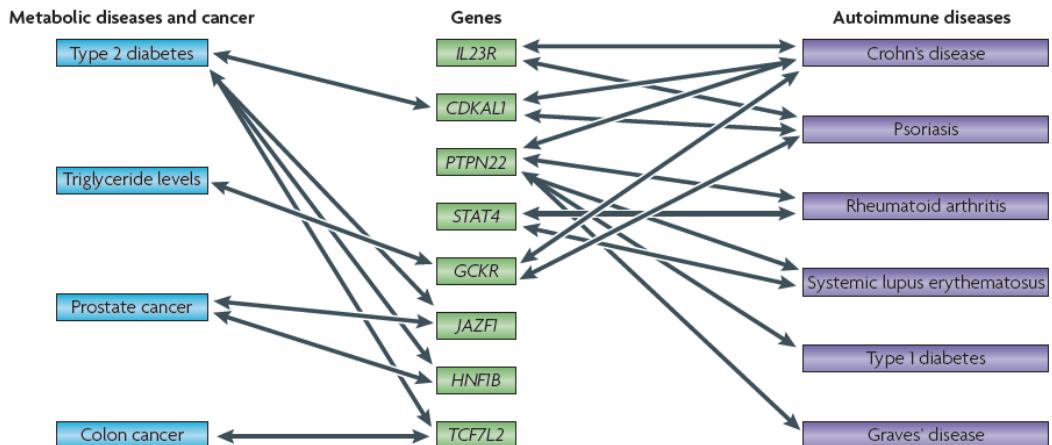


Table 3

Some Recent Genes/Loci Identified in Coronary Artery Disease

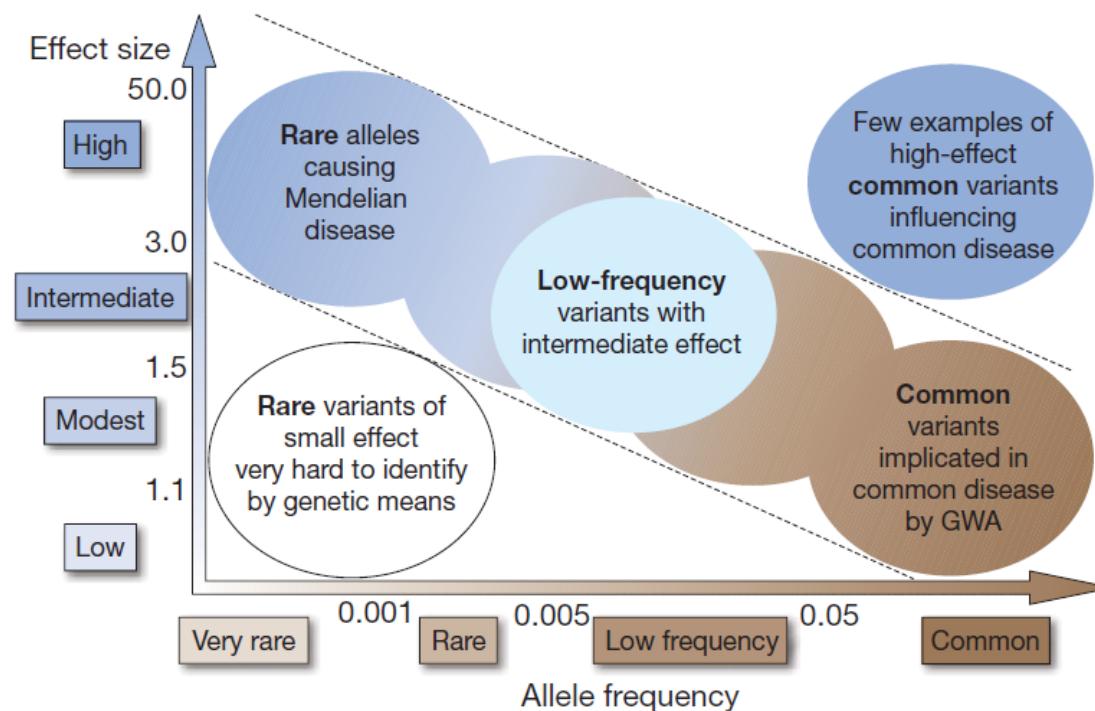
Gene/Locus	Functional Genomics	Independently Replicated	Reference(s)
CFH	Inflammation	Yes	49,50
LTA4H	Inflammation	No	12
FLAP	Inflammation	No	5
Lymphotoxin α	Inflammation	No	69
Galectin 2	Inflammation	No	68
Stromelysin 1	Inflammation	No	61
MHC2TA	Inflammation	No	71
Kalirin	Inflammation	Yes	13
TSP 4	Endothelial integrity	Yes	57-61
Connexin 37	Endothelial integrity	No	61
MEF2A	Endothelial integrity	Yes	62,63
Apo E4	Lipoprotein handling	Yes	52,53,78
LRP6	Lipoprotein handling	No	56
PCSK9	Lipoprotein handling	No	42,43
VAMP8	Thrombosis	No	72
PAI-1	Thrombosis	No	61
Factor V (1691A)	Thrombosis	No	73
Prothrombin (20210A)	Thrombosis	No	73
9p21	Unknown	Yes	38-40

Apo E4 = apolipoprotein E4; FLAP = 5-lipoxygenase activating protein; LRP6 = low-density lipoprotein receptor-related protein 6; LTA4H = leukotriene A4 hydrolase; MEF2A = myocyte enhancer factor 2a; 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.

Common Disease, Common Variant

CDCV, Effect size, and Allele frequency

- “Common Disease, Common Variant” (CDCV) hypothesis:
 - Common variants (SNPs) underlie common diseases/traits (atherosclerotic disease)
 - Why? Evolution: natural selection, fitness & genetic drift
- Effect size vs. Allele frequency
 - Low to intermediate penetrance
 - Low to intermediate **odds ratio (OR) 1.1-1.5**
 - Higher penetrance results in decreased reproductive fitness (unlikely in common diseases!)



What type of study design can you choose?

genome-wide association study



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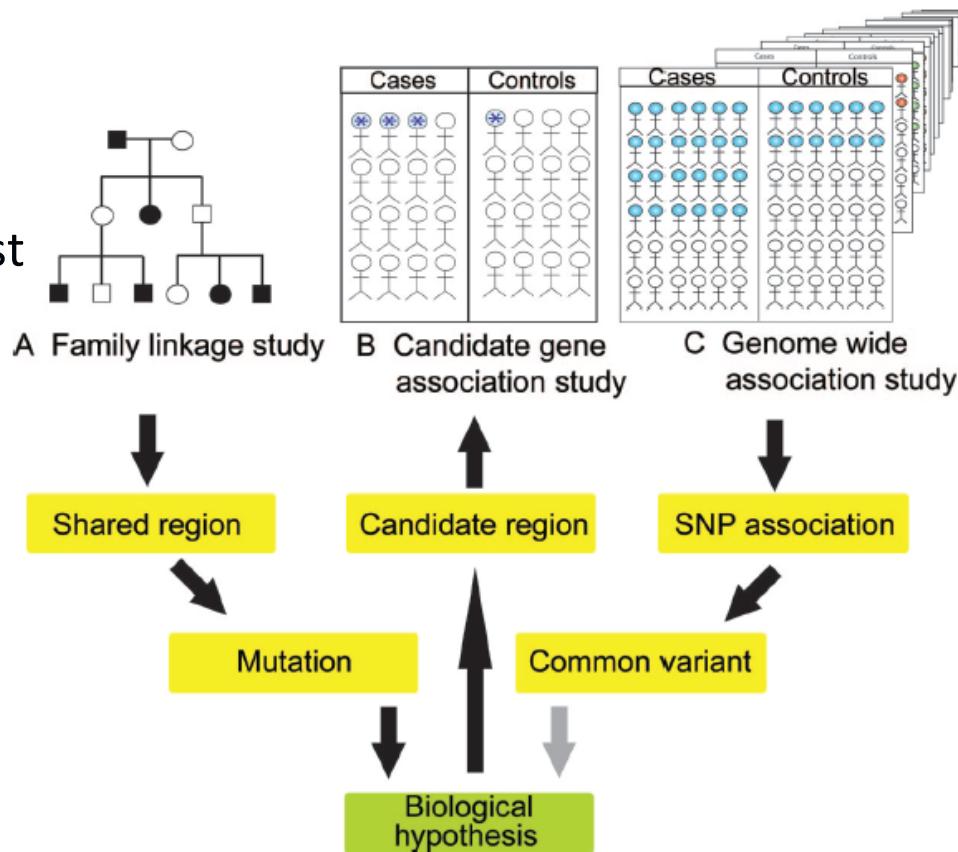
Table 1. Study Designs Used in Genome-wide Association Studies

	Case-Control	Cohort	Trio
Assumptions	Case and control participants are drawn from the same population Case participants are representative of all cases of the disease, or limitations on diagnostic specificity and representativeness are clearly specified Genomic and epidemiologic data are collected similarly in cases and controls Differences in allele frequencies relate to the outcome of interest rather than differences in background population between cases and controls	Participants under study are more representative of the population from which they are drawn Diseases and traits are ascertained similarly in individuals with and without the gene variant	Disease-related alleles are transmitted in excess of 50% to affected offspring from heterozygous parents
Advantages	Short time frame Large numbers of case and control participants can be assembled Optimal epidemiologic design for studying rare diseases	Cases are incident (developing during observation) and free of survival bias Direct measure of risk Fewer biases than case-control studies Continuum of health-related measures available in population samples not selected for presence of disease	Controls for population structure; immune to population stratification Allows checks for Mendelian inheritance patterns in genotyping quality control Logistically simpler for studies of children's conditions Does not require phenotyping of parents
Disadvantages	Prone to a number of biases including population stratification Cases are usually prevalent cases, may exclude fatal or short episodes, or mild or silent cases Overestimate relative risk for common diseases	Large sample size needed for genotyping if incidence is low Expensive and lengthy follow-up Existing consent may be insufficient for GWA genotyping or data sharing Requires variation in trait being studied Poorly suited for studying rare diseases	May be difficult to assemble both parents and offspring, especially in disorders with older ages of onset Highly sensitive to genotyping error

- GWAS is hypothesis-free: no *a priori* ideas on which variant is associated

To hypothesize or not to hypothesize...

- **Family linkage study**
 - Trio-design: parents plus child
- **Candidate Gene Association Study**
 - *A priori* hypothesis
- **Genome-Wide Association Study**
 - No *a priori* hypothesis
 - Cases: some phenotype of interest
 - Controls: random population sample



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, AJHG)
- 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

Anna Helgadottir¹, Andrei Manolescu¹, Gudmar Thorleifsson¹, Solveig Gretarsdottir¹, Helga Jonsdottir¹, Unnur Thorsteinsdottir¹, Nilesh J Samani², Gudmundur Guðmundsson¹, Struan F A Grant¹, Gudmundur Thorgeirsson³, Sigurlaug Sveinbjornsdottir³, Einar M Valdimarsson⁴, Stefan E Matthiasson³, Halldor Johannsson³, Olof Guðmundsdottir¹, Mark E Gurney¹, Jesus Sainz¹, Margret Thorhallsdottir¹, Margaret Andressdottir¹, Michael L Frigge¹, Eric J Topol⁴, Augustine Kong¹, Vilimundur Gudnason⁵, Hakon Hakonarson¹, Jeffrey R Gulcher¹ & Kari Stefansson¹

We mapped a gene predisposing to myocardial infarction to a locus on chromosome 13q12–13. A four-marker single-nucleotide polymorphism (SNP) haplotype in this locus spanning the gene *ALOX5AP* encoding 5-lipoxygenase activating protein (FLAP) is associated with a two times greater risk of myocardial infarction in Iceland. This haplotype also confers almost two times greater risk of stroke. Another *ALOX5AP* haplotype is associated with myocardial infarction in individuals from the UK. Stimulated neutrophils from individuals with myocardial infarction produce more leukotriene B4, a key product in the 5-lipoxygenase pathway, than do neutrophils from controls, and this difference is largely attributed to cells from males who carry the at-risk haplotype. We conclude that variants of *ALOX5AP* are involved in the pathogenesis of both myocardial infarction and stroke by increasing leukotriene production and inflammation 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

Anna Helgadottir,^{1*} Gudmar Thorleifsson,^{1*} Andrei Manolescu,^{1*} Solveig Gretarsdottir,¹ Thorarinn Blöndal,¹ Aslaug Jonasdottir,¹ Adalbjorg Jonasdottir,¹ Asgeir Sigurdsson,¹ Adam Baker,¹ Amar Palsson,¹ Gisli Masson,¹ Daniel F. Gudbjartsson,¹ Kristinn P. Magnusson,¹ Karl Andersen,² Allan I. Levey,³ Valgerdur M. Backman,¹ Sigurborg Matthiasdottir,¹ Thorbjorg Jonsdottir,¹ Stefan Palsson,¹ Helga Einarsdottir,¹ Steinunn Gunnarsdottir,¹ Arnaldur Gylfason,¹ Viola Vaccarino,³ W. Craig Hooper,³ Muredach P. Reilly,⁴ Christopher B. Granger,⁵ Harland Austin,³ Daniel J. Rader,⁴ Svti H. Shah,⁵ Arshed A. Quyyumi,³ Jeffrey R. Gulcher,¹ Gudmundur Thorgeirsson,² Unnur Thorsteinsdottir,¹ Augustine Kong,^{1,†} Kari Stefansson^{1,†}

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

Wellcome Trust Case-Control Consortium

University Medical Center
Utrecht

- 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**

Vol 447 | 7 June 2007 | doi:10.1038/nature05911

nature

ARTICLES

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

The Wellcome Trust Case Control Consortium*

One famous example

9p21

- 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

Anna Helgadottir,^{1,*} Gudmar Thorleifsson,^{1,*} Andrei Manolescu,^{1,*} Solveig Gretarsdottir,¹ Thorarinn Blonadal,¹ Aslaug Jonasdottir,¹ Adalbjorg Jonasdottir,¹ Asgeir Sigurdsson,¹ Adam Baker,¹ Amar Palsson,¹ Gisli Masson,¹ Daniel F. Gudbjartsson,¹ Kristinn P. Magnusson,¹ Karl Andersen,² Allan I. Levey,³ Valgerdur M. Backman,¹ Sigurborg Matthiassdottir,¹ Thorbjorg Jonsdottir,¹ Stefan Palsson,¹ Helga Einarsdottir,¹ Steinunn Gunnarsdottir,¹ Amaldur Gylfason,¹ Viola Vaccarino,³ W. Craig Hooper,³ Muredach P. Reilly,⁴ Christopher B. Granger,⁵ Harland Austin,³ Daniel J. Rader,⁴ Svti H. Shah,⁵ Arshed A. Quyyumi,³ Jeffrey R. Gulcher,¹ Gudmundur Thorgeirsson,² Unnur Thorsteinsdottir,¹ Augustine Kong,^{1,†} Kari Stefansson¹

A Common Allele on Chromosome 9 Associated with Coronary Heart Disease

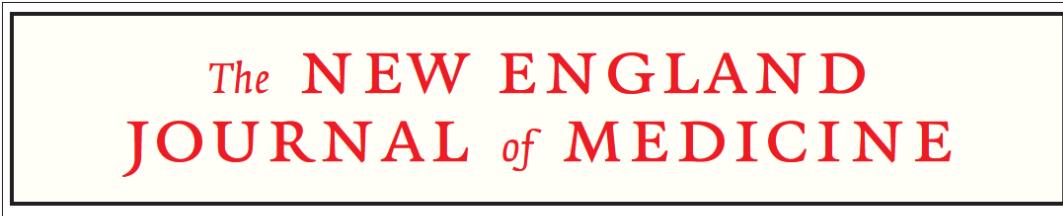
Ruth McPherson,^{1,*†} Alexander Pertsemlidis,^{2,*} Nihan Kavaslar,¹ Alexandre Stewart,¹ Robert Roberts,¹ David R. Cox,³ David A. Hinds,³ Len A. Pennacchio,^{4,5} Anne Tybjaerg-Hansen,⁶ Aaron R. Folsom,⁷ Eric Boerwinkle,⁸ Helen H. Hobbs,^{2,9} Jonathan C. Cohen^{2,10†}

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



Genomewide Association Analysis of Coronary Artery Disease

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nature

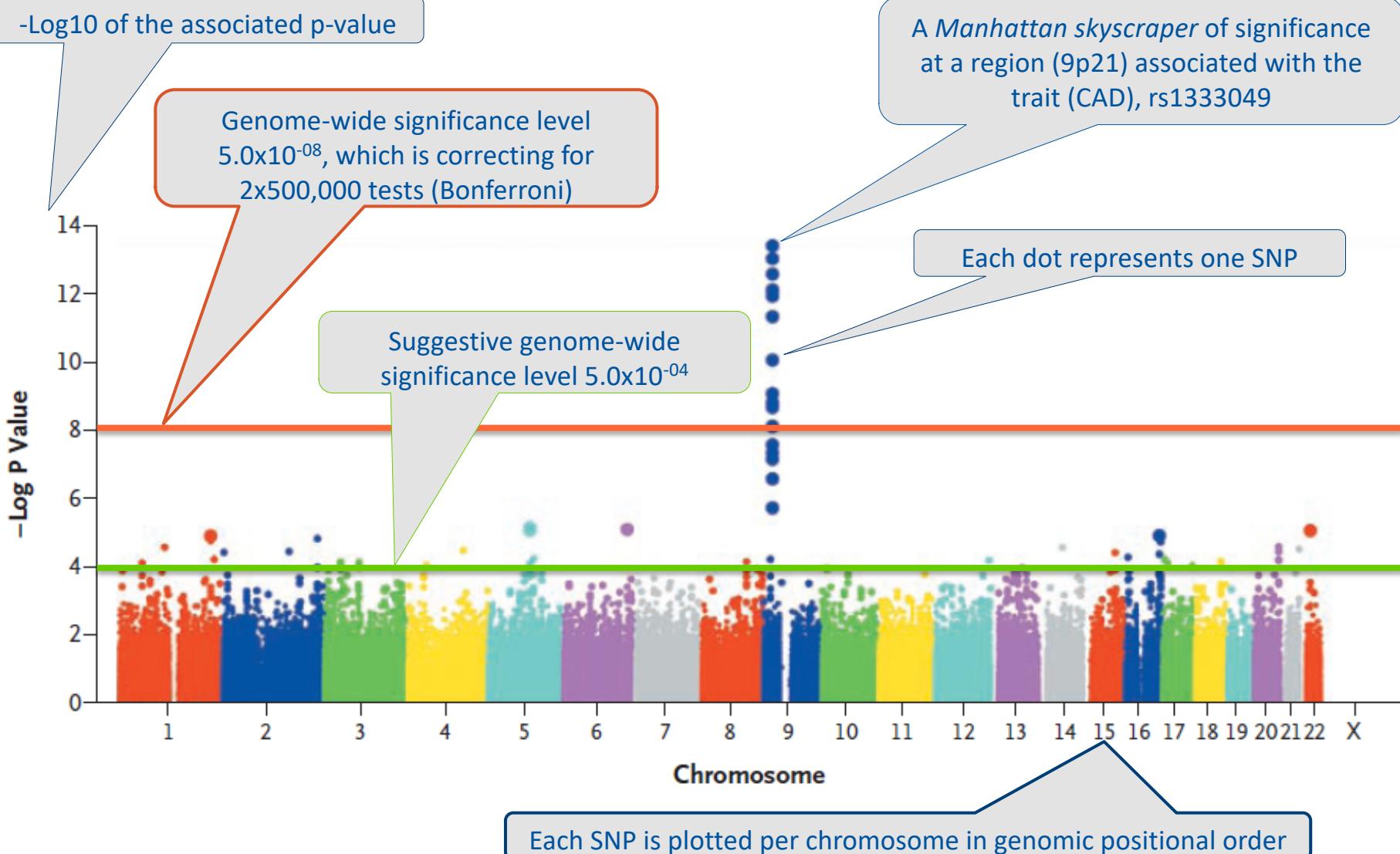
ARTICLES

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

The Wellcome Trust Case Control Consortium*

One famous example

9p21 in the WTCCC

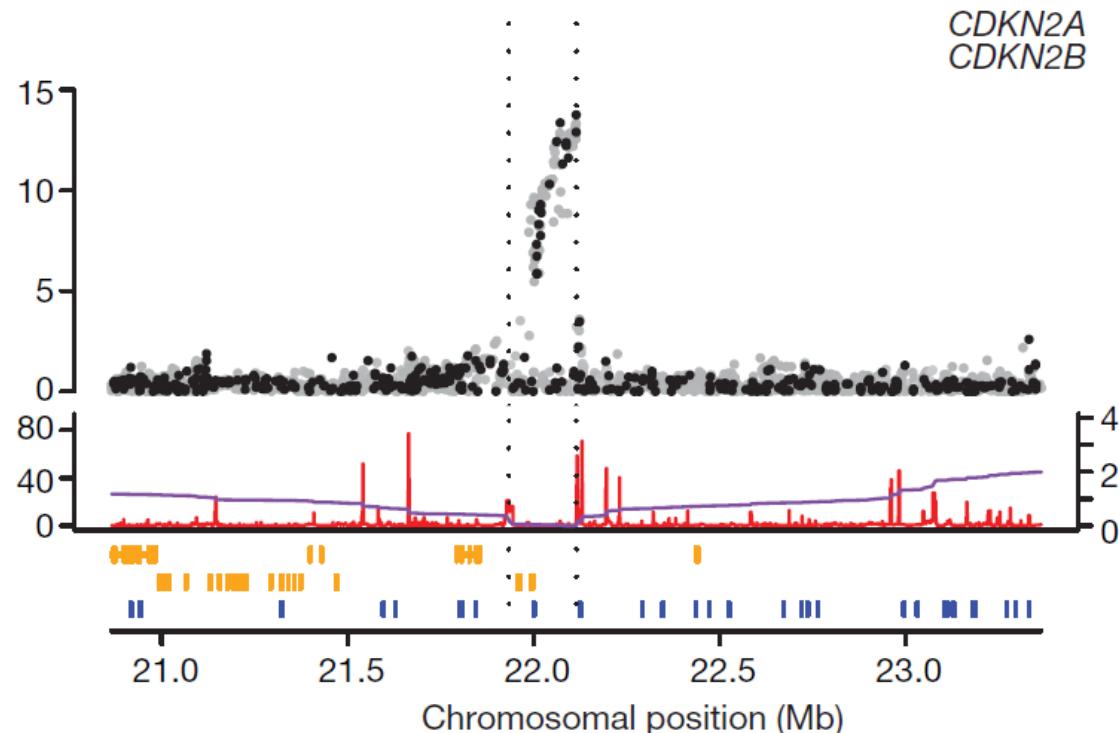


One famous example

9p21 a closer look

- The SNPs 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

CAD hit region, chromosome 9



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	$\log_{10}(BF)$, additive	$\log_{10}(BF)$, 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}	Standard analysis	1.16×10^{-13}	11.66	11.19	C	C	1.47 (1.27-1.70)	1.9 (1.61-2.24)	0.474	0.554

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

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

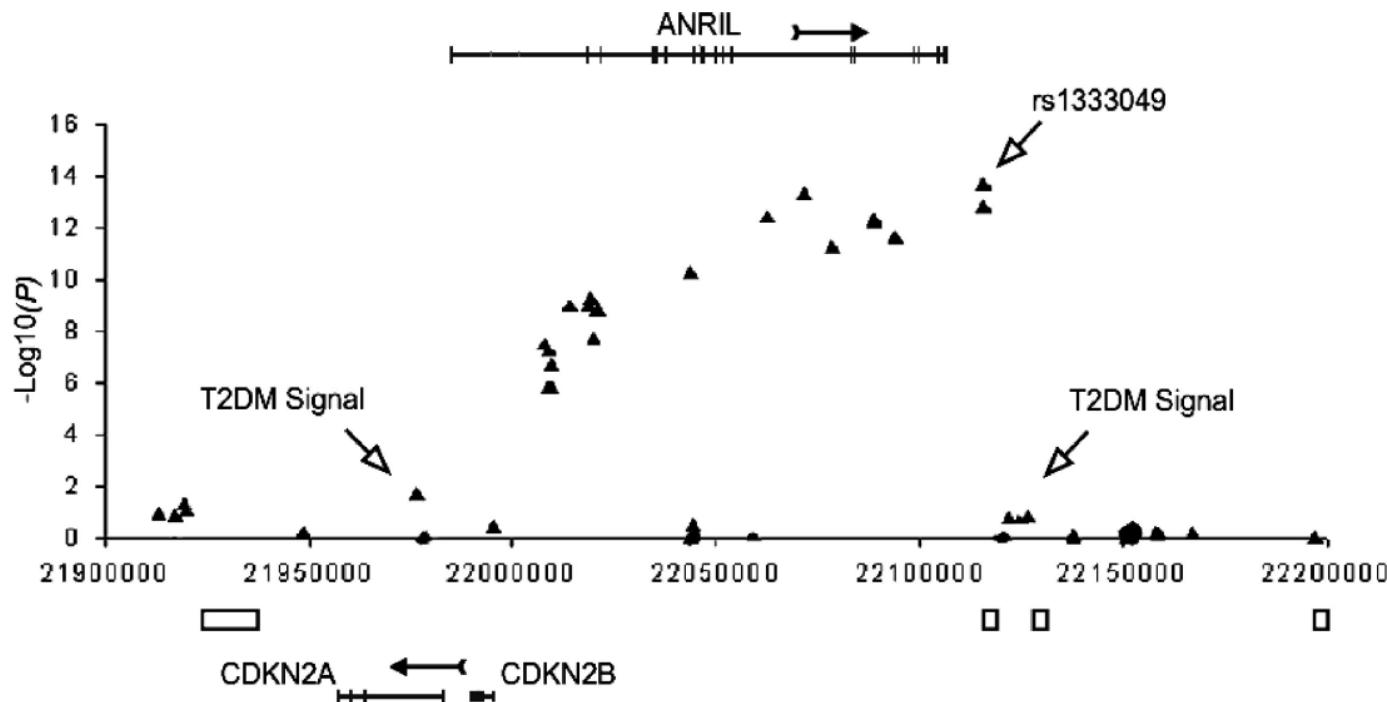
Risk allele: minor allele
Odds ratio: the odds of exposure between cases and controls

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

9p21 points to a RNA gene

how does this explain an acute phenomenon like MI?

- 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)



9p21 is 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
- Clear and concise report

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

FRAMINGHAM RISK SCORING ALGORITHMS FEMALE SPECIFIC																																																																																																																																																																																	
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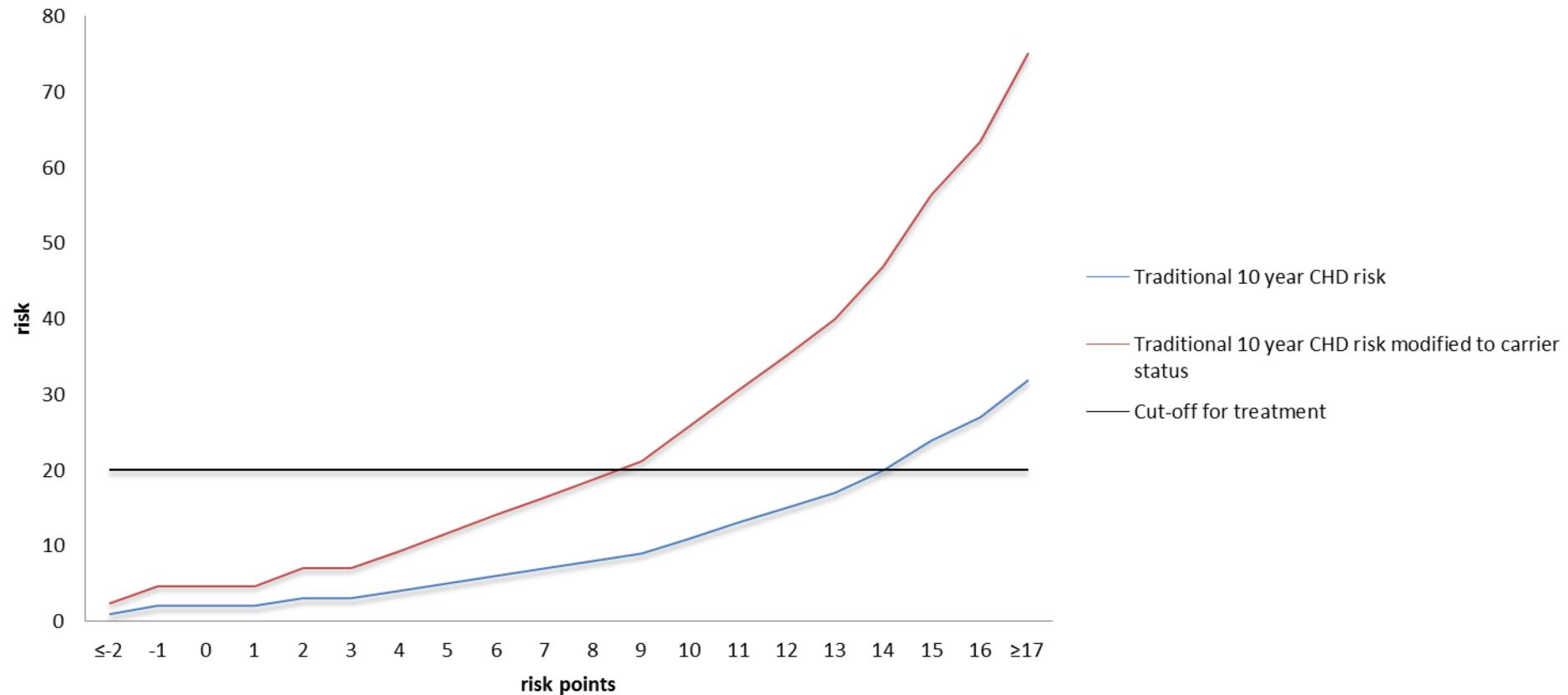
This test was developed and its performance characteristics determined by the deCODE genetics Diagnostic Laboratory. It has not been cleared or approved by the U. S. Food and Drug Administration (FDA) deCODE Diagnostics Laboratory – Testing Site: St. Lucie & 101 Raynfield, Isla Vista Customer Service: 15700 W. 103rd St. Suite 200, Lemoore, CA 93649 – Phone: (833) 783-9998 – www.decodediagnostics.com ©2009 deCODE genetics Diagnostic Laboratory All rights reserved – document version 2.1

Points Total	10 Year CHD Risk Traditional	Reclassified MI Risk Factor	10 Year CHD Risk Modified
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0	=	2 % x	2.35 =
1	=	2 % x	2.35 =
2	=	3 % x	2.35 =
3	=	3 % x	2.35 =
4	=	4 % x	2.35 =
5	=	5 % x	2.35 =
6	=	6 % x	2.35 =
7	=	7 % x	2.35 =
8	=	8 % x	2.35 =
9	=	9 % x	2.35 =
10	=	11 % x	2.35 =
11	=	13 % x	2.35 =
12	=	15 % x	2.35 =
13	=	17 % x	2.35 =
14	=	20 % x	2.35 =
15	=	24 % x	2.35 =
16	=	27 % x	2.35 =
≥17	=	≥32 % x	2.35 =

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





University Medical Center
Utrecht

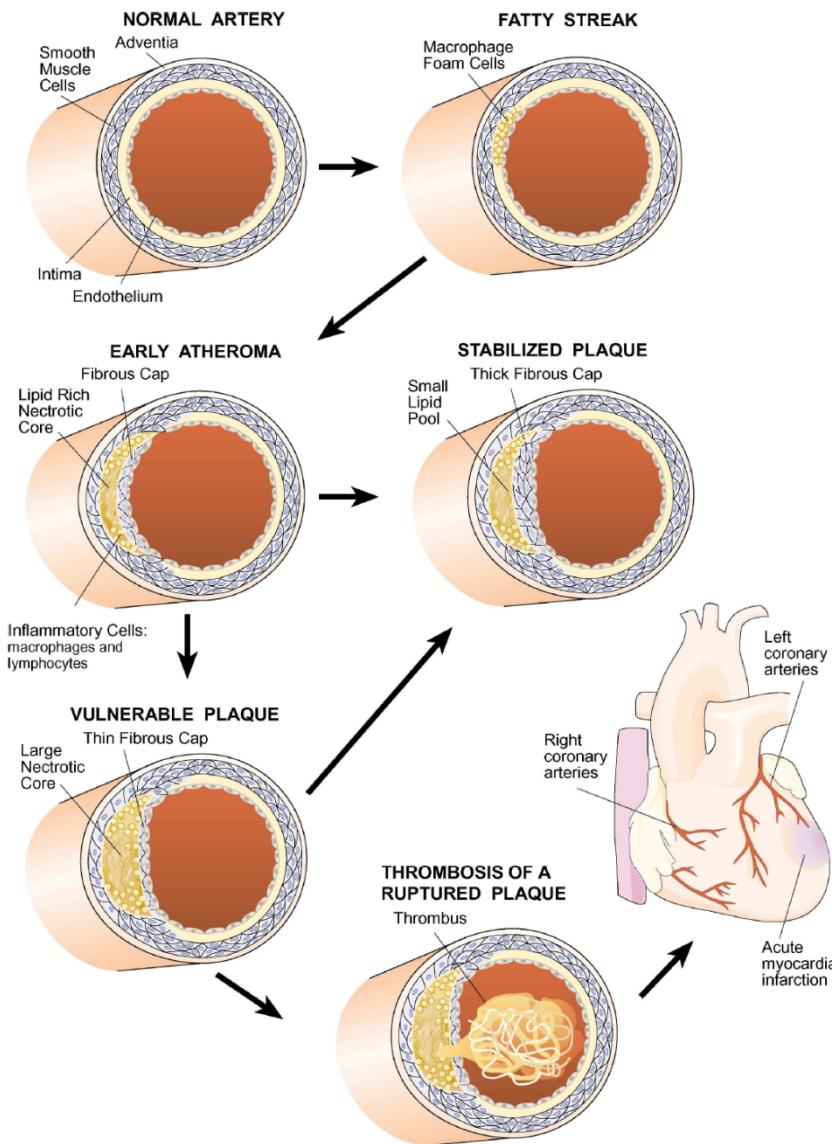
Genetics of Advanced Atherosclerotic Disease

GWAS OF PLAQUE PHENOTYPES



Atherosclerosis & Cardiovascular Disease

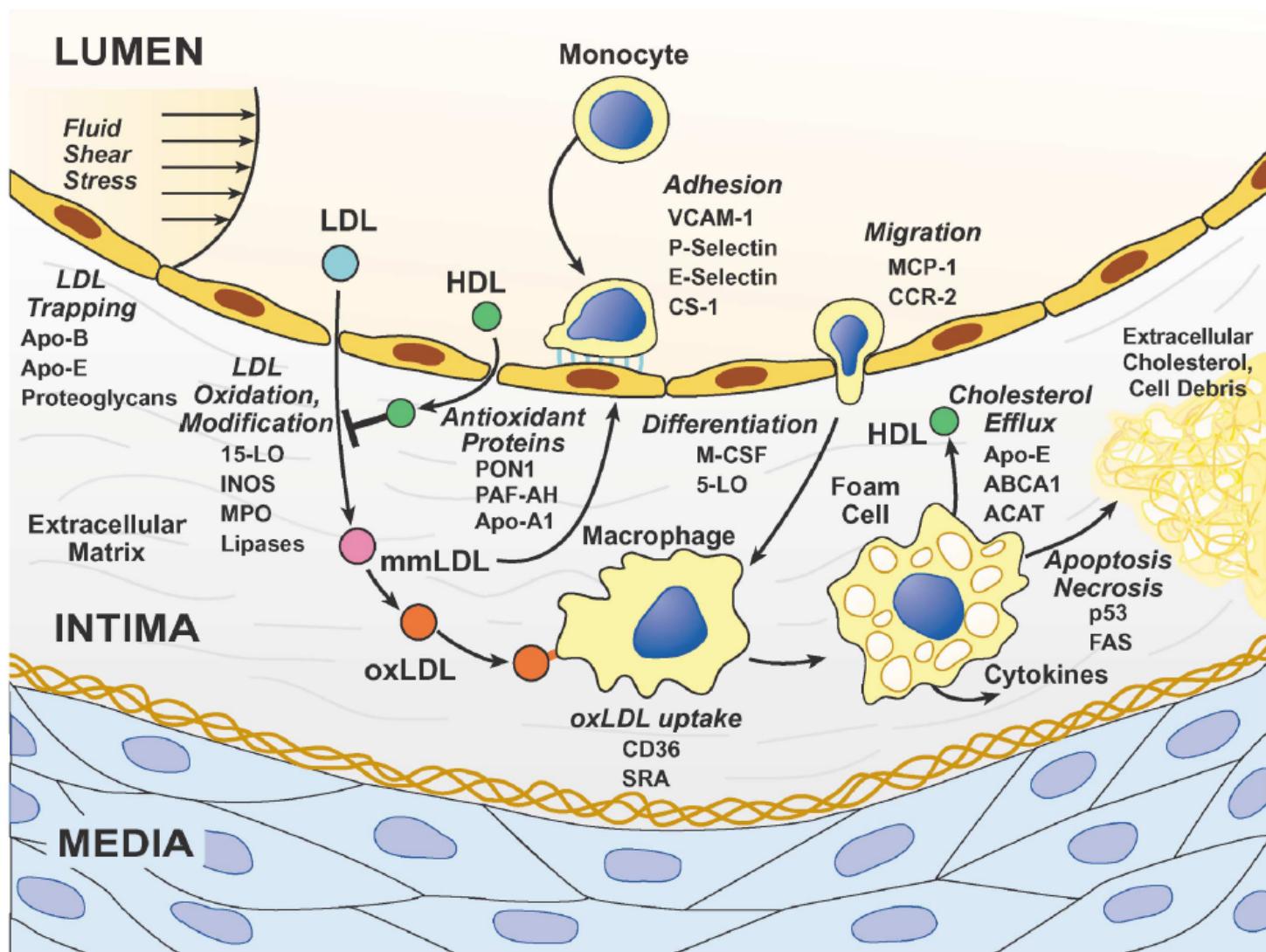
a very complex process and disease



Atherosclerosis & Cardiovascular Disease

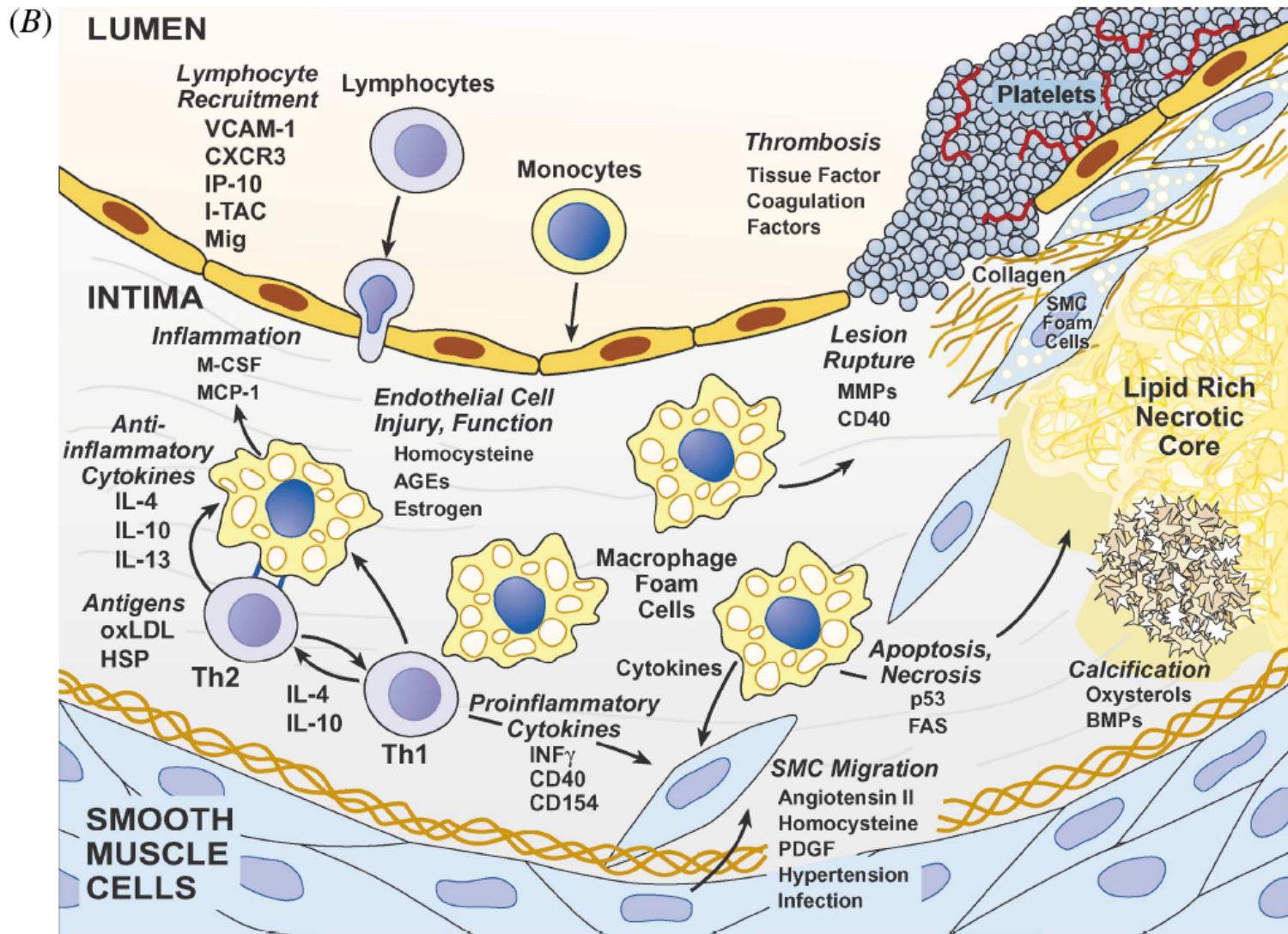
a very complex process and disease

(A)



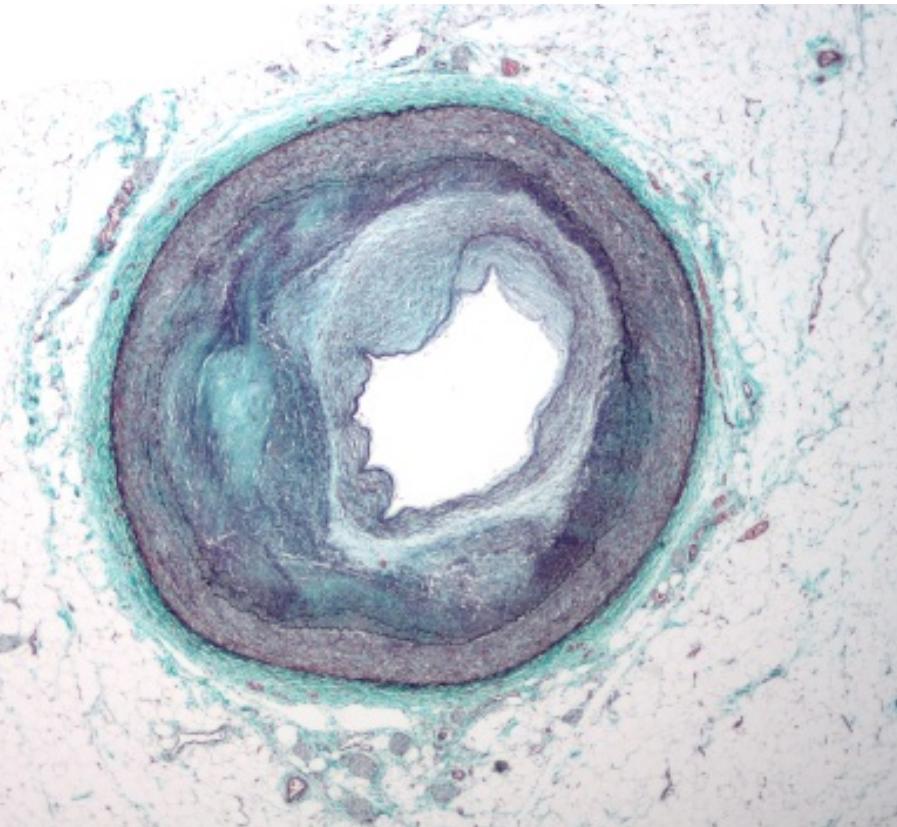
Atherosclerosis & Cardiovascular Disease

a very complex process and disease



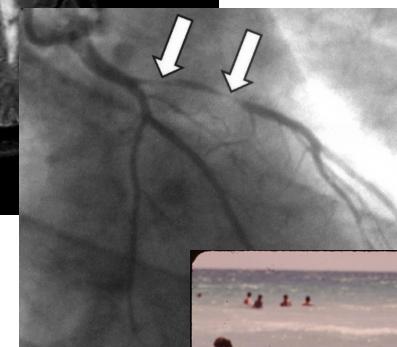
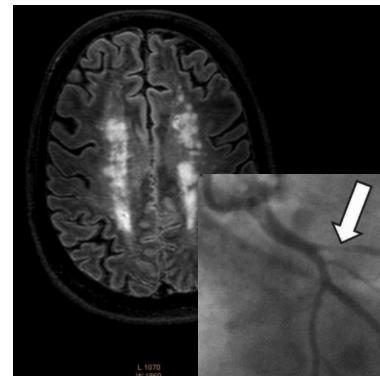
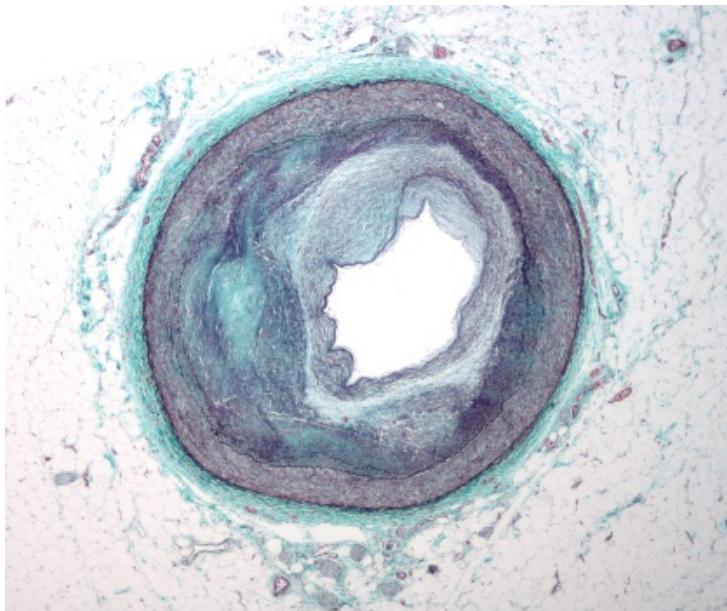
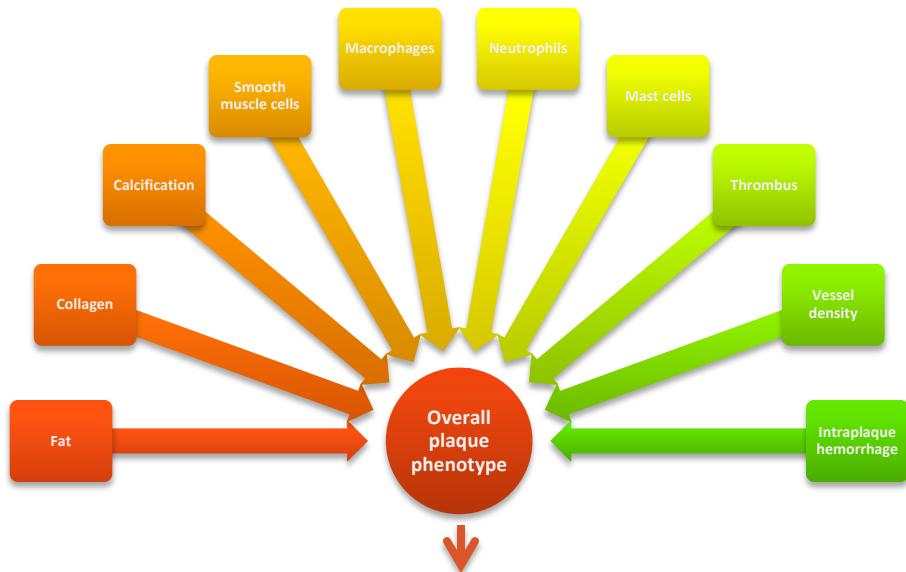
Atherosclerosis causes cardiovascular disease

- *Atherosclerosis* is a chronic inflammation of the arteries and the underlying cause of *cardiovascular disease*
- Histologically *atherosclerosis* can be defined by *plaque phenotypes*



- Fat
- Collagen
- Calcification
- Smooth muscle cell (SMC) content
- Macrophage content
- Neutrophil content
- Mast cell content
- Thrombus presence
- Intraplaque hemorrhage
- Vessel density
- Overall plaque phenotype

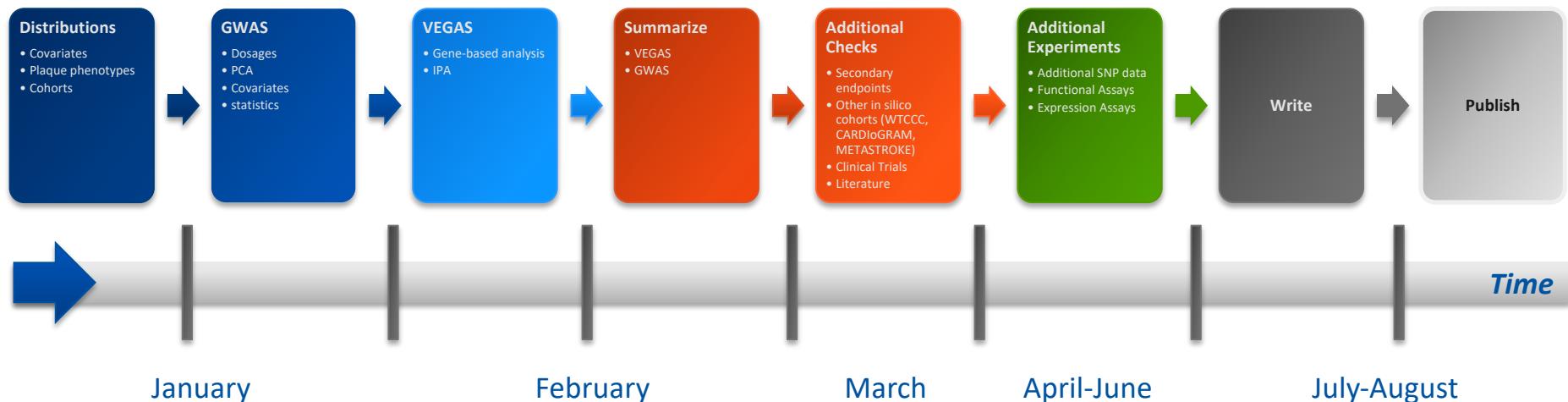
The plaque is the sum of its parts



Summarizing Steps to Take

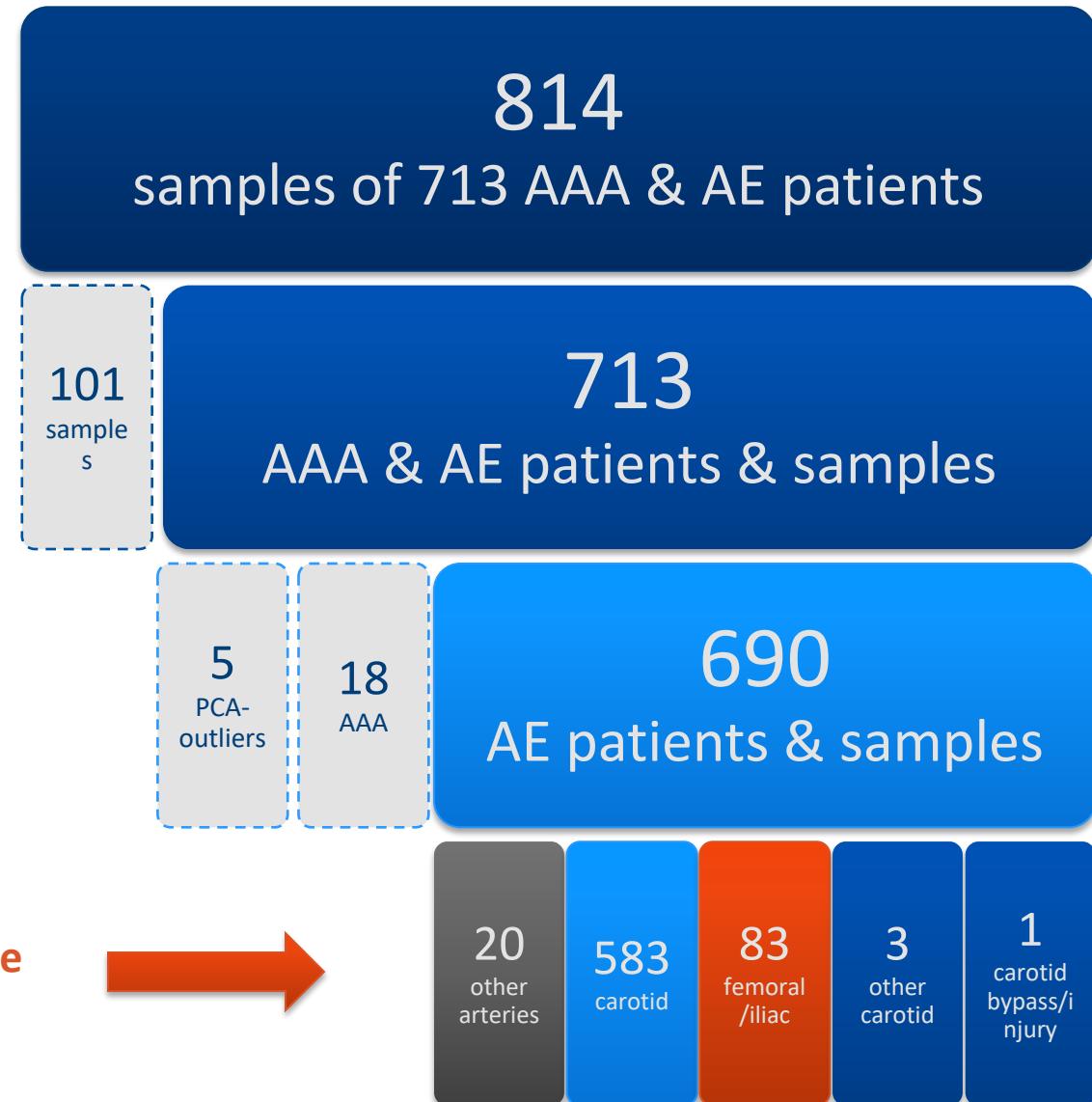
timeline is in constant flux

- To ensure progress and quality there will be *regular meetings*
 - Genetics & Statistics:* Paul de Bakker, Jessica van Setten, Folkert Asselbergs
 - Athero-Express:* Gerard Pasterkamp



Breakdown of samples & patients

- 814 samples of 713 patients
 - Exclude 101 duplicate samples
 - Exclude 18 AAA
 - Exclude 5 PCA-outliers
 - 690 patients can be used (AE)
 - 583 carotid arteries (CEA)
 - 83 femoral arteries (FEA)
 - 4 other carotid artery surgeries
 - 20 other artery surgeries

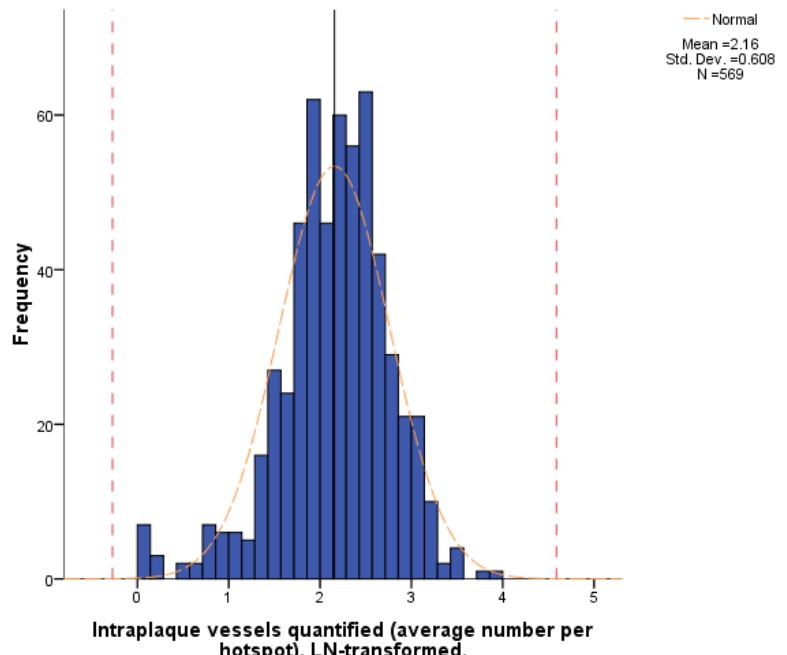


Analyses: Focus on CEA, also analyze FEA and AE



Vessel density as an interesting start

- Previously associated with outcome



Vascular Medicine

Composition of Carotid Atherosclerotic Plaque Is Associated With Cardiovascular Outcome

A Prognostic Study

Willem E. Hellings, MD, PhD*; Wouter Peeters, MD*; Frans L. Moll, MD, PhD; Sebastiaan R.D. Piers; Jessica van Setten, BSc; Peter J. Van der Spek, PhD; Jean-Paul P.M. de Vries, MD, PhD; Kees A. Seldenrijk, MD, PhD; Peter C. De Bruin, MD, PhD; Aryan Vink, MD, PhD; Evelyn Velema, BSc; Dominique P.V. de Kleijn, PhD; Gerard Pasterkamp, MD, PhD

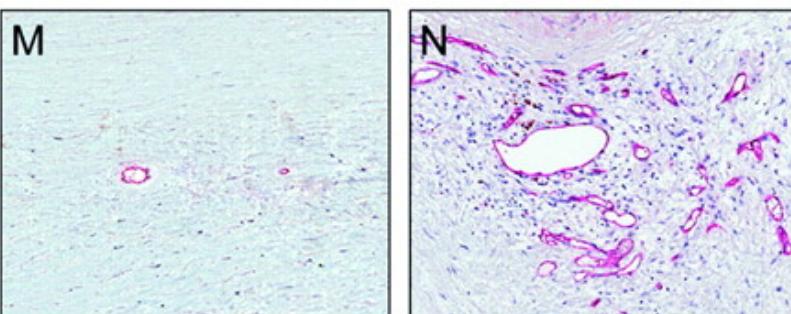
Background—Identification of patients at risk for primary and secondary manifestations of atherosclerotic disease progression is based mainly on established risk factors. The atherosclerotic plaque composition is thought to be an important determinant of acute cardiovascular events, but no prospective studies have been performed. The objective of the present study was to investigate whether atherosclerotic plaque composition is associated with the occurrence of future vascular events.

Methods and Results—Atherosclerotic carotid lesions were collected from patients who underwent carotid endarterectomy and were subjected to histological examination. Patients underwent clinical follow-up yearly, up to 3 years after carotid endarterectomy. The primary outcome was defined as the composite of a vascular event (vascular death, nonfatal stroke, nonfatal myocardial infarction) and vascular intervention. The cumulative event rate at 1-, 2-, and 3-year follow-up was expressed by Kaplan-Meier estimates, and Cox proportional hazards regression analyses were performed to assess the independence of histological characteristics from general cardiovascular risk factors. During a mean follow-up of 2.3 years, 196 of 818 patients (24%) reached the primary outcome. Patients whose excised carotid plaque revealed plaque hemorrhage or marked intraplaque vessel formation demonstrated an increased risk of primary outcome (risk difference=30.6% versus 17.2%; hazard ratio [HR] with [95% confidence interval]=1.7 [1.2 to 2.5]; and risk difference=30.0% versus 23.8%; HR=1.4 [1.1 to 1.9], respectively). Macrophage infiltration (HR=1.1 [0.8 to 1.5]), large lipid core (HR=1.1 [0.7 to 1.6]), calcifications (HR=1.1 [0.8 to 1.5]), collagen (HR=0.9 [0.7 to 1.3]), and smooth muscle cell infiltration (HR=1.3 [0.9 to 1.8]) were not associated with clinical outcome. Local plaque hemorrhage and increased intraplaque vessel formation were independently related to clinical outcome and were independent of clinical risk factors and medication use.

Conclusions—The local atherosclerotic plaque composition in patients undergoing carotid endarterectomy is an independent predictor of future cardiovascular events. (*Circulation*. 2010;121:1941-1950.)

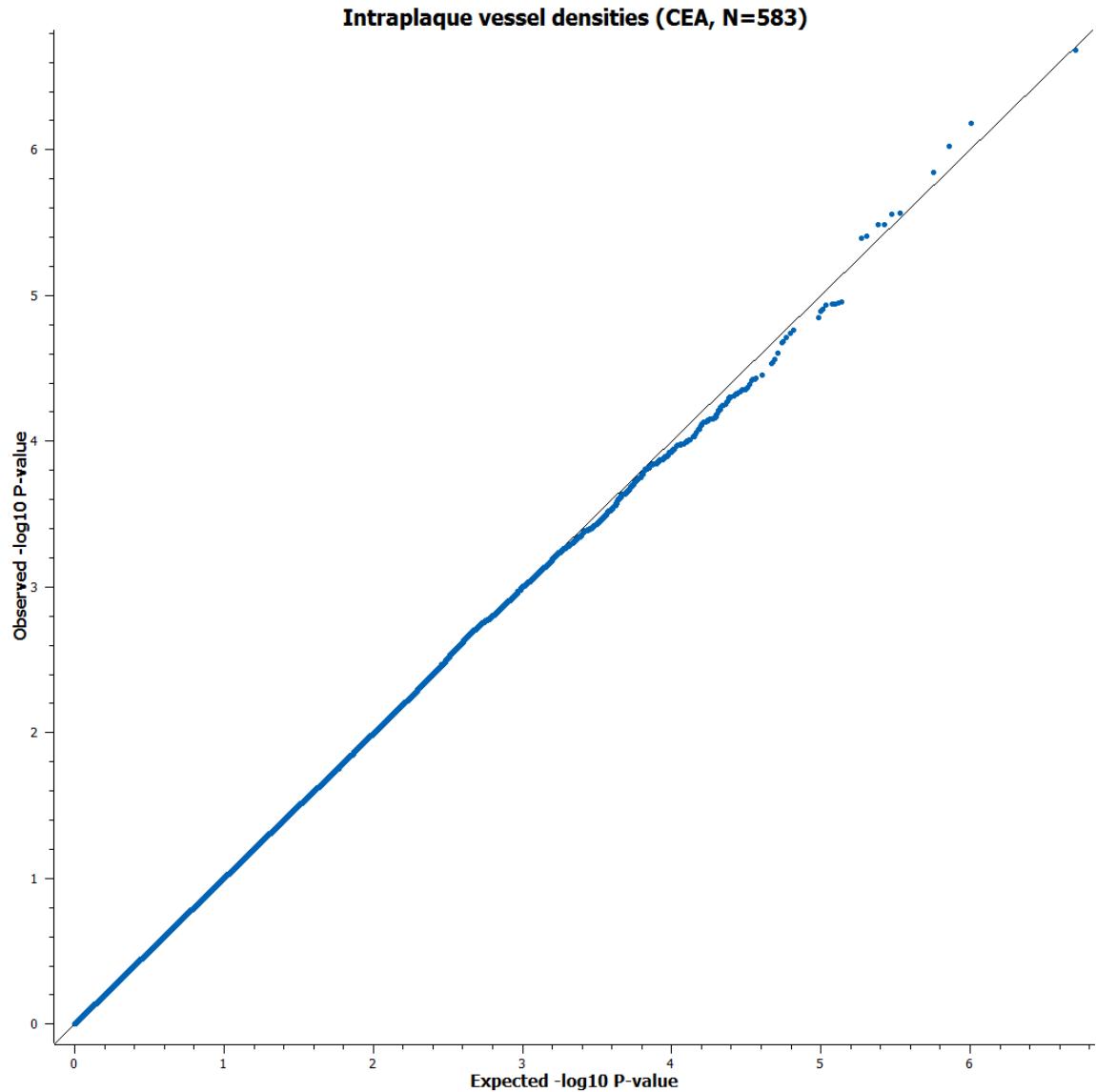
Key Words: atherosclerosis ■ cardiovascular diseases ■ outcome assessment ■ carotid arteries
■ hemorrhage ■ blood vessels

Intraplaque vessels



Preliminary *genome-wide significance of less frequent, high quality SNPs*

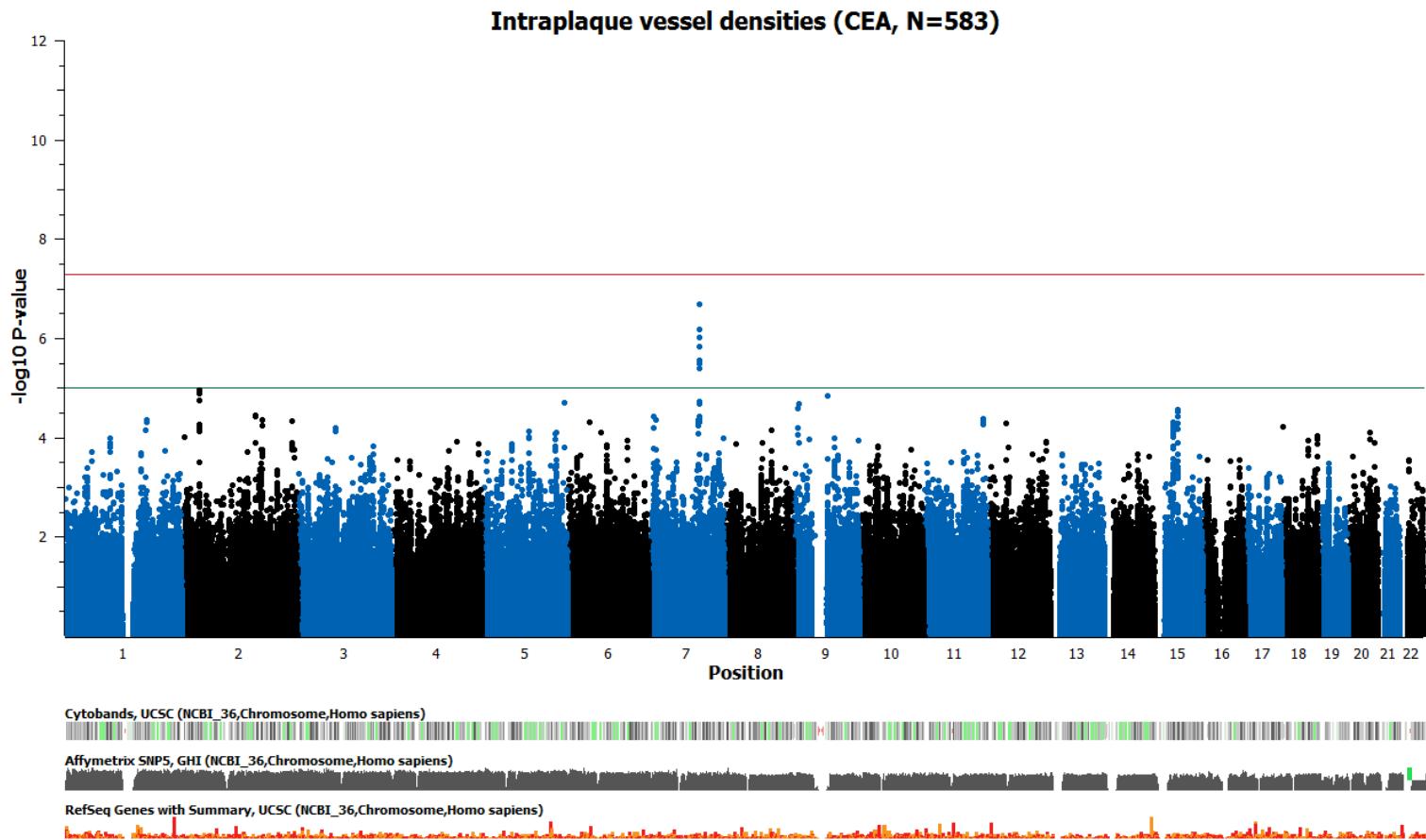
- MAF >3%
- INFO-metric > 0.90
- Still with good lambda



Preliminary

genome-wide significance of less frequent, high quality SNPs

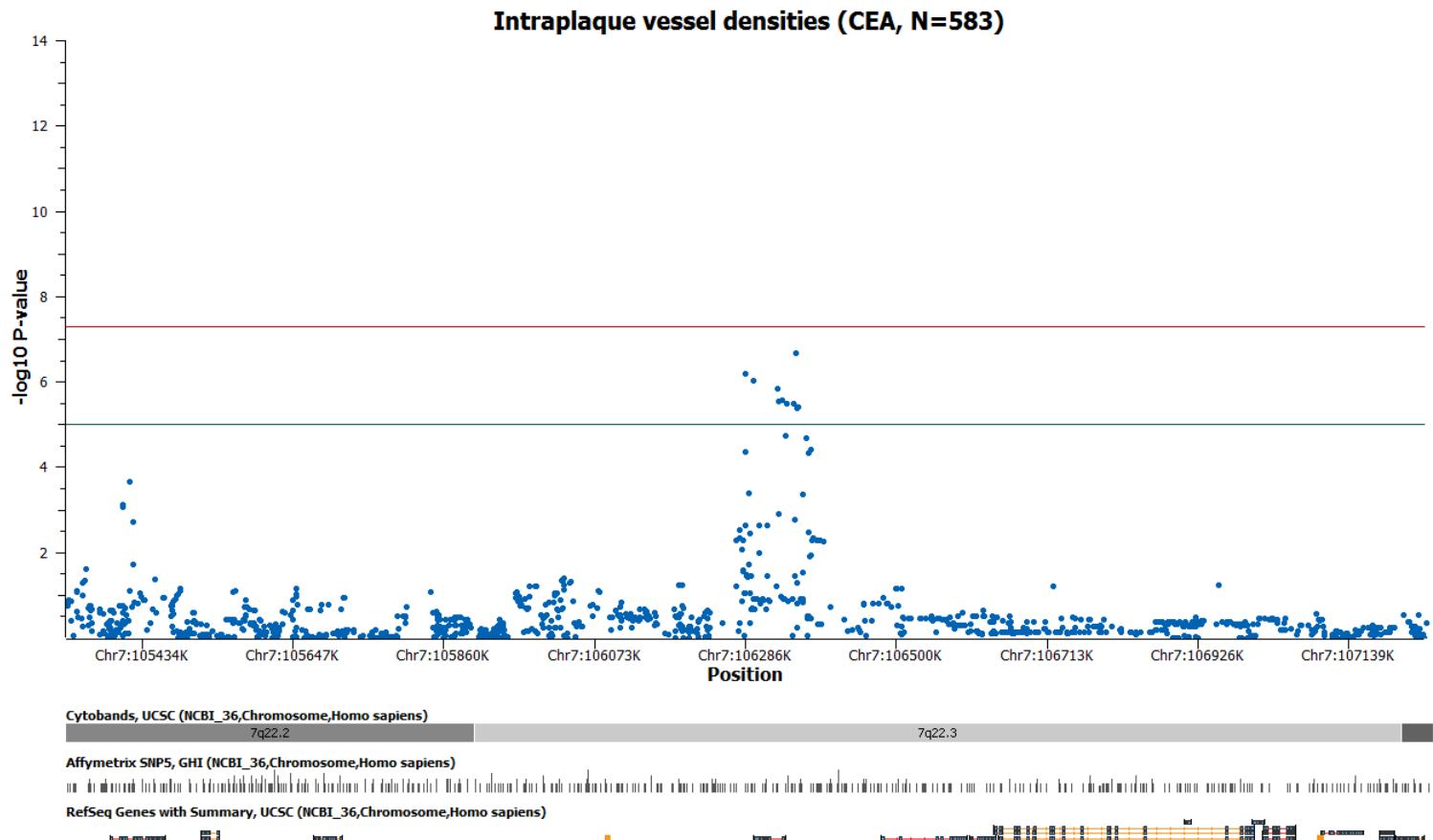
- No genome-wide significance
- One suggestively significant hit
- Look into further: genes, literature



Preliminary

genome-wide significance of less frequent, high quality SNPs

- Chromosome 7
- No imputation artefact? No genotyping error? Case distribution? Minor allele frequency?





University Medical Center
Utrecht

Genetic Burden of Disease (Risk)

GENETIC BURDEN SCORES



CARDIoGRAM Study

- Coronary Artery Disease Genome–Wide Replication And Meta–Analysis Study: CARDIoGRAM
- > 20,000 cases and > 60,000 controls
 - Myocardial infarction (MI), coronary artery disease (CAD) or both
 - CAD: MI, CABG, PTCA, AP
 - Age limit: 45–66
- Recent studies uncovered around 13 variants associated with MI/CAD
- Sample size greatly influences power and effect size to discover new variants
- CARDIoGRAM sought to solves this issue
- 13 novel susceptibility loci for CAD were discovered

nature
genetics

Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease

We performed a meta-analysis of 14 genome-wide association studies of coronary artery disease (CAD) comprising 22,233 individuals with CAD (cases) and 64,762 controls of European descent followed by genotyping of top association signals in 56,682 additional individuals. This analysis identified 13 loci newly associated with CAD at $P < 5 \times 10^{-8}$ and confirmed the association of 10 of 12 previously reported CAD loci. The 13 new loci showed risk allele frequencies ranging from 0.13 to 0.91 and were associated with a 6% to 17% increase in the risk of CAD per allele. Notably, only three of the new loci showed significant association with traditional CAD risk factors and the majority lie in gene regions not previously implicated in the pathogenesis of CAD. Finally, five of the new CAD risk loci appear to have pleiotropic effects, showing strong association with various other human diseases or traits.

It has been estimated that heritable factors account for 30%–60% of the inter-individual variation in the risk of coronary artery disease (CAD)¹. Recently, genome-wide association studies (GWAS) have identified several common variants that associate with risk of CAD². However, in aggregate, these variants explain only a small fraction of the heritability of CAD, probably partly due to the limited power of previous studies to discover effects of modest size. Recognizing the need for larger studies, we formed the transatlantic Coronary Artery Disease Genome-wide Replication and Meta-analysis (CARDIoGRAM) consortium³. We performed a meta-analysis of 14 GWAS of CAD comprising 22,233 cases and 64,762 controls, all of European ancestry (Supplementary Table 1a–c and Supplementary Fig. 1). We then genotyped the lead SNPs within the most promising previously unidentified loci as well as a subset of previously reported CAD loci in up to 56,682 additional subjects (approximately half cases and half controls) (Supplementary Table 2a,b). Lastly, we explored potential mechanisms and intermediate pathways by which previously unidentified loci may mediate risk.

Nine of the twelve loci previously associated with CAD through individual GWAS achieved genome-wide significance ($P < 5 \times 10^{-8}$) in our initial meta-analysis (Table 1 and Supplementary Table 3). We were, however, unable to test the previously reported association with a haplotype and a rare SNP in *LPA* in our GWAS data^{4,5}, but we observed robust association with the rare *LPA* variant in our replication samples through direct genotyping (Table 1).

Thus, 10 of the 12 loci previously associated with CAD at a genome-wide significance level surpassed the same threshold of significance in CARDIoGRAM.

We selected 23 new loci with a significance level of $P < 5 \times 10^{-6}$ in the meta-analysis for follow up (Online Methods and Supplementary Note). Taking the number of loci into consideration, our replication study had >90% power to detect effect sizes observed in the GWAS meta-analysis. Of the 23 loci, 13 replicated using our *a priori* definition of a validated locus, that is, showing independent replication after Bonferroni correction and also achieving $P < 5 \times 10^{-8}$ in the combined discovery and replication data (Table 2, Fig. 1 and Supplementary Figs. 2 and 3). Results for all loci from the replication phase are shown in Supplementary Tables 4 and 5.

The 13 new loci had risk allele frequencies ranging from 0.13 to 0.91 and were associated with a 6% to 17% increase in the risk of CAD per allele (Table 2). Out of the 13 new loci, the additive model appeared most appropriate for 6 whereas the recessive model performed best at 5 and the dominant model at 2 loci (Supplementary Table 6).

In sub-group analyses, 20 out of 22 loci with $P < 5 \times 10^{-8}$ (known and new loci combined; for one locus, age subgroups were not available) had higher odds ratios for early onset than for late onset CAD ($P = 1.2 \times 10^{-4}$ for observed versus expected; Supplementary Table 7). The CAD loci showed consistent associations irrespective of case definition, although the odds ratios for most individual SNPs tended to be slightly greater for cases with angiographically proven CAD than for cases with unknown angiographic status ($P = 0.019$ for observed versus expected) (Supplementary Table 8). In contrast, subgroup analyses in males and females revealed no sex-specific effects for any risk alleles (Supplementary Table 7) or for their observed versus expected pattern of association ($P = 0.4$).

Among 7,637 CAD cases and 7,523 controls for whom we had individual level genotype data, the minimum and maximum number of risk alleles observed per individual was 15 and 37, respectively, when considering 23 CAD susceptibility loci. The mean weighted risk score was significantly higher for cases than for controls ($P < 10^{-20}$). Furthermore, being in the top tenth percentile or lowest tenth percentile of the weighted score was associated with an odds ratio for CAD of 1.88 (95% CI 1.67–2.11) and 0.55 (95% CI 0.48–0.64), respectively, compared to the fiftieth percentile. The change in odds ratio for CAD across a broader spectrum of categories of the weighted score is shown in Supplementary Figure 4.

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Replication & Discovery

- 10 out of 12 previously associated loci could be replicated
- 13 novel loci were uncovered

Table 1 Association evidence in CARDioGRAM for previously published loci for coronary disease (previously reported with genome-wide significance, $P < 5 \times 10^{-8}$)

Band	SNP	Gene(s) in region	n	Risk allele frequency (risk allele)	CARDioGRAM		Reference
					OR (95% CI)	P	
1p32.3	rs11206510 ^a	<i>PCSK9</i>	102,352	0.82 (T)	1.08 (1.05–1.11)	9.10×10^{-8}	1.15 (1.10–1.21) ²⁶
1p13.3	rs599839 ^b	<i>SORT1</i>	83,873	0.78 (A)	1.11 (1.08–1.15)	2.89×10^{-10}	1.29 (1.18–1.40) ²¹
1q41	rs17465637 ^c	<i>MIA3</i>	25,197	0.74 (C)	1.14 (1.09–1.20)	1.36×10^{-8}	1.20 (1.12–1.30) ²¹
2q33.1	rs6725887 ^b	<i>WDR12</i>	77,954	0.15 (C)	1.14 (1.09–1.19)	1.12×10^{-9}	1.16 (1.10–1.22) ²⁶
3q22.3	rs2306374 ^b	<i>MRAS</i>	77,843	0.18 (C)	1.12 (1.07–1.16)	3.34×10^{-8}	1.15 (1.11–1.19) ²³
6p24.1	rs12526453 ^b	<i>PHACTR1</i>	83,050	0.67 (C)	1.10 (1.06–1.13)	1.15×10^{-9}	1.13 (1.09–1.17) ²⁶
6q25.3	rs3798220 ^d	<i>LPA</i>	32,584	0.02 (C)	1.51 (1.33–1.70)	3.00×10^{-11}	1.92 (1.48–2.49) ⁵
9p21.3	rs4977574 ^b	<i>CDKN2A</i> , <i>CDKN2B</i>	84,256	0.46 (G)	1.29 (1.23–1.36)	1.35×10^{-22}	1.25 (1.18–1.31), 1.37 (1.26–1.48) ^{20,21,27,28}
10q11.21	rs1746048 ^a	<i>CXCL12</i>	136,416	0.87 (C)	1.09 (1.07–1.13)	2.93×10^{-10}	1.33 (1.20–1.48) ²¹
12q24.12	rs3184504 ^b	<i>SH2B3</i>	67,746	0.44 (T)	1.07 (1.04–1.10)	6.35×10^{-6}	1.13 (1.08–1.18) ³⁸
19p13.2	rs1122608 ^b	<i>LDLR</i>	49,693	0.77 (G)	1.14 (1.09–1.18)	9.73×10^{-10}	1.14 (1.09–1.19) ²⁶
21q22.11	rs9982601 ^b	<i>MRPS6</i>	46,230	0.15 (T)	1.18 (1.12–1.24)	4.22×10^{-10}	1.19 (1.13–1.27) ²⁶

Data taken from ^athe combined analysis, ^bthe meta-analysis, ^conly genotyped data from a subset of studies and ^dthe replication.

Table 2 New loci for coronary disease

Band	SNP	Gene(s) in region	Risk allele frequency (risk allele)	Meta-analysis		Replication		Combined analysis	
				P	n	P	n	OR (95% CI)	P
1p32.2	rs17114036	<i>PPAP2B</i>	0.91 (A)	1.43×10^{-8}	80,870	3.18×10^{-12}	52,356	1.17 (1.13–1.22)	3.81×10^{-19}
6p21.31	rs17609940	<i>ANKS1A</i>	0.75 (G)	2.21×10^{-6}	83,997	1.18×10^{-3}	53,415	1.07 (1.05–1.10)	1.36×10^{-8}
6q23.2	rs12190287	<i>TCF21</i>	0.62 (C)	4.64×10^{-11}	78,290	3.25×10^{-4}	52,598	1.08 (1.06–1.10)	1.07×10^{-12}
7q32.2	rs15156924	<i>ZC3HC1</i>	0.62 (C)	2.22×10^{-9}	80,011	7.37×10^{-10}	54,189	1.09 (1.07–1.12)	9.18×10^{-18}
9q34.2	rs579459	<i>ABO</i>	0.21 (C)	1.16×10^{-7}	77,138	7.02×10^{-8}	46,840	1.10 (1.07–1.13)	4.08×10^{-14}
10q24.32	rs12413409	<i>CYP17A1</i> , <i>CNNM2</i> , <i>NT5C2</i>	0.89 (G)	1.47×10^{-6}	80,940	1.38×10^{-4}	48,801	1.12 (1.08–1.16)	1.03×10^{-9}
11q23.3	rs964184	<i>ZNF259</i> , <i>APOA5</i> – <i>A4-C3-A1</i>	0.13 (G)	8.02×10^{-10}	82,562	2.20×10^{-9}	52,930	1.13 (1.10–1.16)	1.02×10^{-17}
13q34	rs4773144	<i>COL4A1</i> , <i>COL4A2</i>	0.44 (G)	4.15×10^{-7}	77,113	1.31×10^{-3}	37,618	1.07 (1.05–1.09)	3.84×10^{-9}
14q32.2	rs2895811	<i>HHIP1</i>	0.43 (C)	2.67×10^{-7}	63,184	4.59×10^{-5}	51,054	1.07 (1.05–1.10)	1.14×10^{-10}
15q25.1	rs3825807	<i>ADAMTS7</i>	0.57 (A)	9.63×10^{-6}	80,849	1.39×10^{-8}	48,803	1.08 (1.06–1.10)	1.07×10^{-12}
17p13.3	rs216172	<i>SMG6</i> , <i>SRR</i>	0.37 (C)	6.22×10^{-7}	57,238	2.11×10^{-4}	54,303	1.07 (1.05–1.09)	1.15×10^{-9}
17p11.2	rs12936587	<i>RASDI1</i> , <i>SMCR3</i> , <i>PEMT</i>	0.56 (G)	4.89×10^{-7}	76,952	1.35×10^{-4}	52,648	1.07 (1.05–1.09)	4.45×10^{-10}
17q21.32	rs46522	<i>UBE2Z</i> , <i>GIP</i> , <i>ATP5G1</i> , <i>SNF8</i>	0.53 (T)	3.57×10^{-6}	83,867	8.88×10^{-4}	53,766	1.06 (1.04–1.08)	1.81×10^{-8}

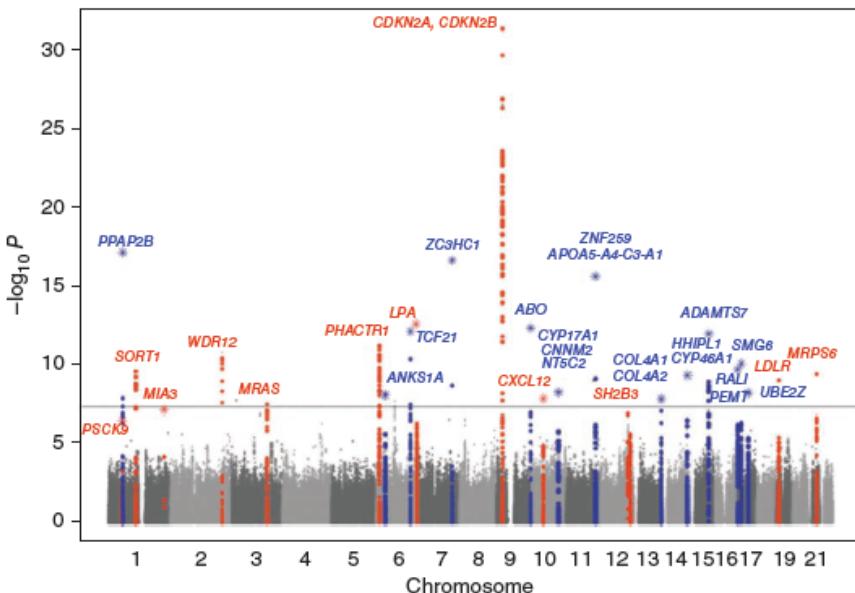


Figure 1 Graphical summary (Manhattan plot) of genome-wide association results. The x axis represents the genome in physical order; the y axis shows $-\log_{10} P$ for all SNPs. Data from the discovery phase are shown in circles, and data from the combined discovery and replication phases are shown in stars. Genes at the significant loci are listed above the signals. Known loci are shown in red and newly discovered loci are shown in blue.

Pleiotropic effects of discovered loci association with risk factors and other diseases

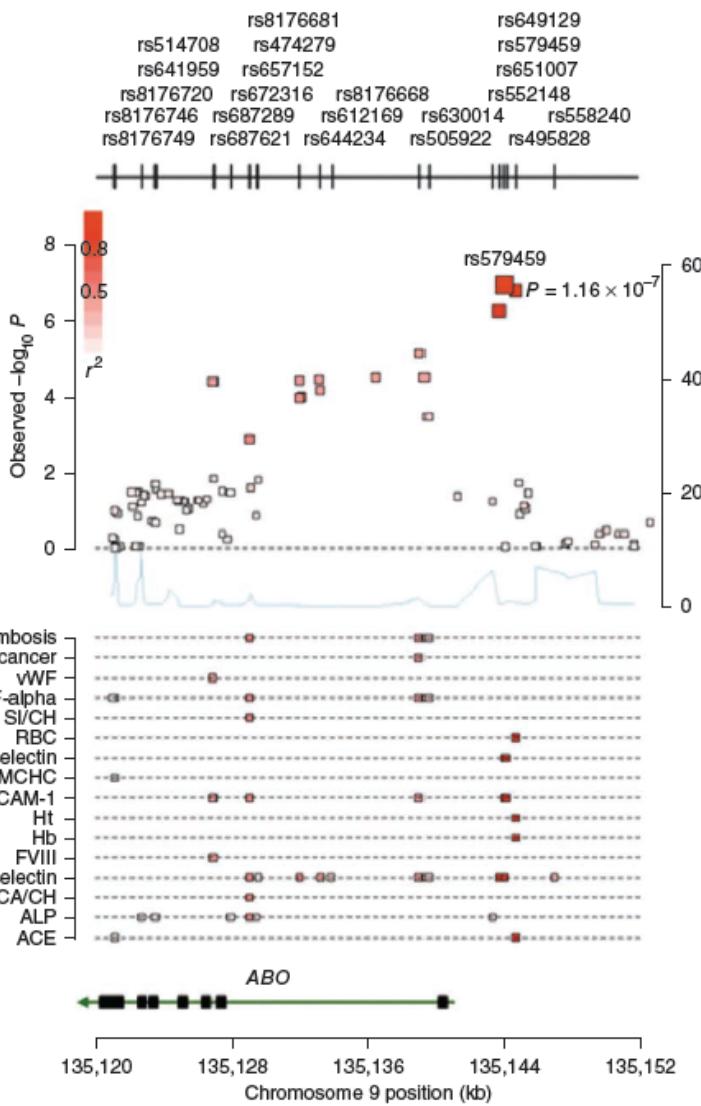
Table 3 Effects of new CAD loci on traditional risk factors in combined analysis of ARIC and KORA F3 and F4 ($n = 13,171$)

SNP	Band	Gene(s) in region	Phenotype	β (95% CI) ^a	P
rs579459	9q34.2	<i>ABO</i>	Total cholesterol	1.720 (0.554–2.885)	0.0038
			LDL cholesterol	1.538 (0.468–2.608)	0.0049
rs12413409	10q24.32	<i>CYP17A1, CNNM2, NT5C2</i>	Hypertension	0.141 (0.044–0.238)	0.0043
rs964184 ^b	11q23.3	<i>ZNF259, APOA5-A4-C3-A1</i>	HDL cholesterol	-1.926 (-2.441 to -1.411)	2.28×10^{-13}
			Total cholesterol	4.578 (3.191–5.964)	9.84×10^{-11}
			LDL cholesterol	1.699 (0.417–2.980)	0.0094

Results from fixed-effects meta-analysis based on β coefficients and standard errors from linear (for total, LDL and HDL cholesterol) and logistic (for hypertension) regression analysis of the single studies for which meta-analytic $P < 0.01$. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

^aEstimated pooled regression coefficients with 95% confidence intervals. Cholesterol levels are in mg/dl. ^bPrevious genome-wide studies have demonstrated strong association of rs964184 with triglycerides³⁹.

Figure 2 Example of overlapping association signals for multiple traits at the *ABO* gene region on chromosome 9q34. In the upper panel, the association signal for coronary disease at the *ABO* gene region in CARDIoGRAM and the positions and rs numbers of SNPs in this region are shown. The size of the boxes illustrates the number of individuals available for this respective SNP. In the lower panel, all SNPs with P values at the genome-wide significance level of $P < 5 \times 10^{-8}$ based on the National Human Genome Research Institute GWAS catalog (accessed on 28 June 2010) for all diseases and traits are shown. The degree of linkage disequilibrium (r^2) between the lead SNPs for coronary disease and the other traits is reflected by the color of the squares (upper panel) and the small bars (lower panel), from dark red (high LD) to faint red (low LD). SI/CH, sitosterol normalized to cholesterol; CA/CH, campesterol normalized to cholesterol; ALP, alkaline phosphatase; ACE, angiotensin converting enzyme; FVIII, coagulation factor VIII; vWF, von Willebrand factor.



Weighted Polygenic Burden Score

- TraitX is a risk factor and/or (status) marker and associated with atherosclerotic diseases and/or phenotypes
- Hypothesis:** A weighted polygenic burden score based on SNPs associated with TraitX (GBS TraitX) is associated with atherosclerotic disease
- Validation:** A GBS TraitX is associated with natural log-transformed TraitX
- Question:** Is a GBS TraitX associated with:
 - Plaque phenotype
 - Plaque protein expression
 - (secondary) clinical events
 - Symptoms prior to surgery
 - Or any other relevant (clinical) feature?

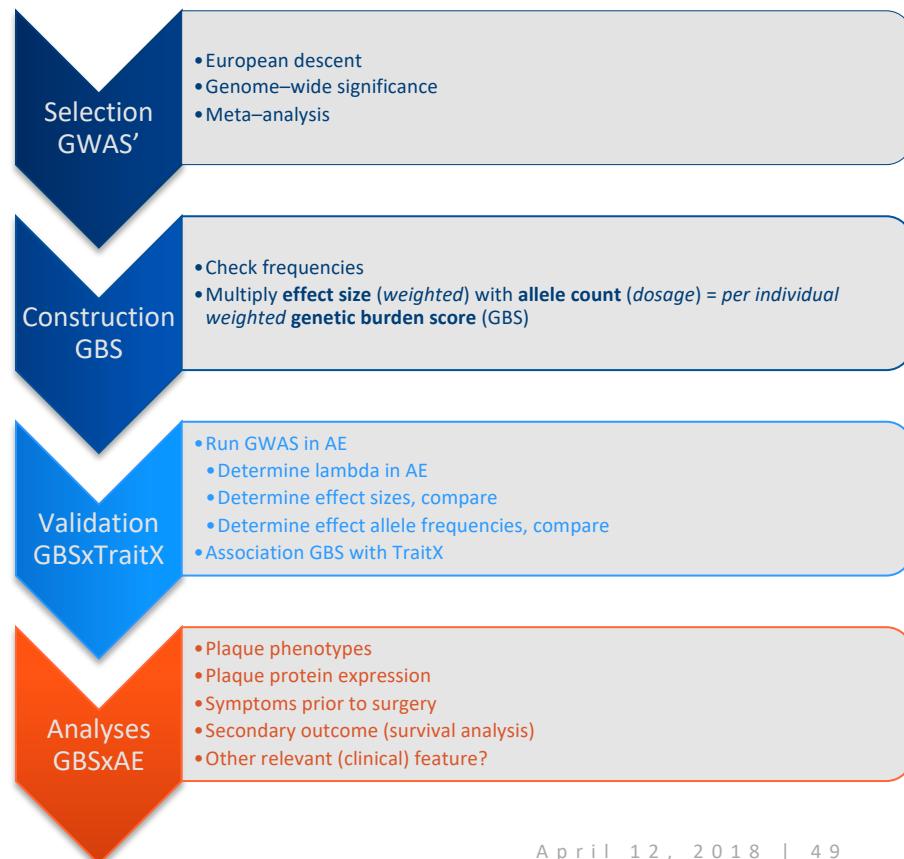
Weighted Polygenic Burden Score

$$\sum_n^i \beta_i \times SNP_n$$

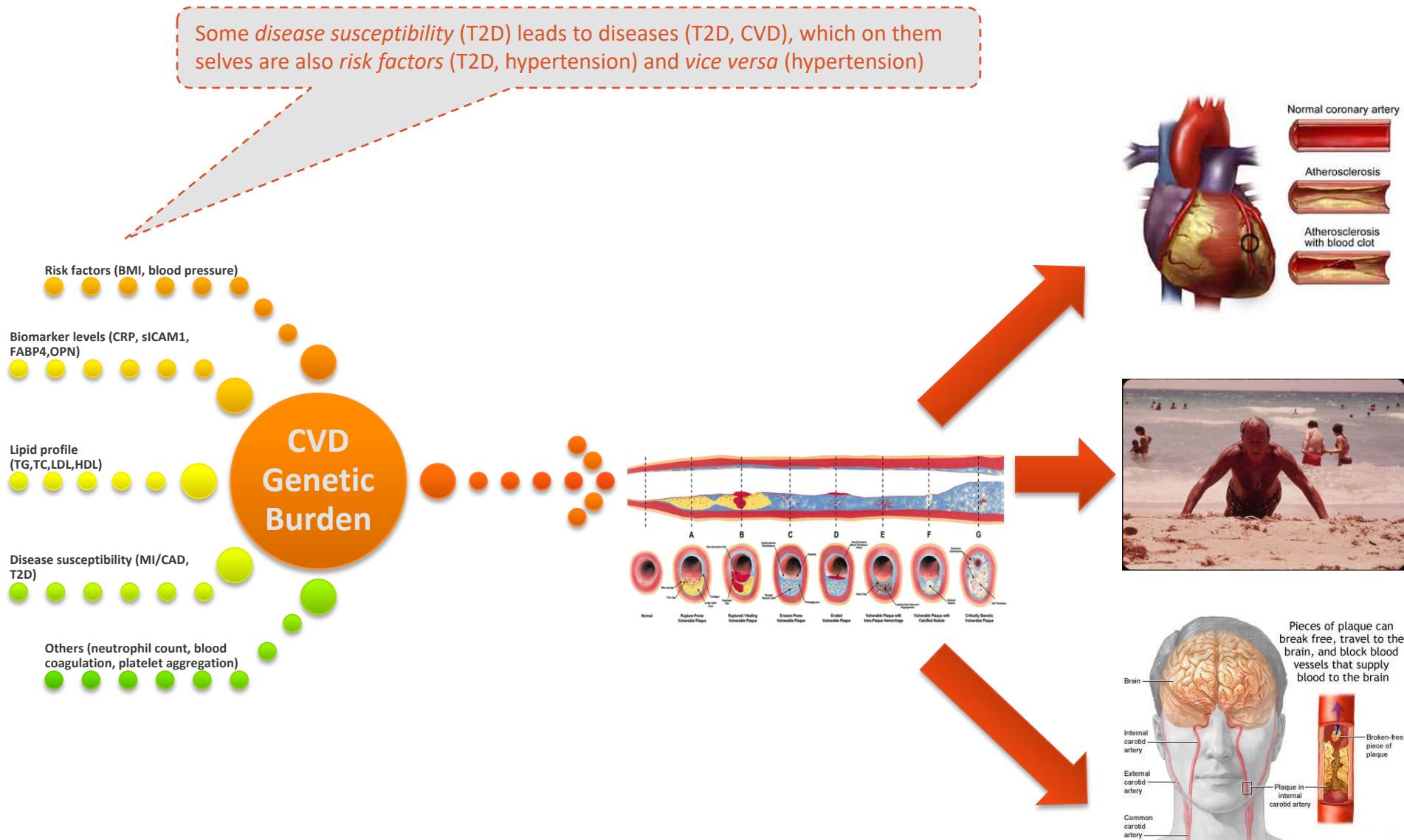
i =effect size (β) at locus

n =number alleles at locus (range: 0 to 2), i.e. dosage

SNP =locus proxy



Genetic Burden to Atherosclerotic disease



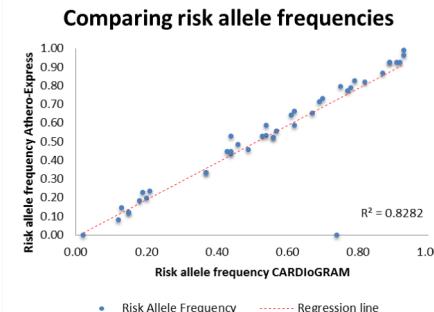
MI & CAD in the Athero-Express

- Similar to CARDIoGRAM
- CAD cases
 - (fatal) MI, AP, PTCA, CABG prior to surgery (self-reported) or during follow-up
- Controls: all CAD-free patients
- Age limits: <45, <50, <60, <66 years → focus on CAD <60 years (similar to CARDIoGRAM)

	CAD <45	CAD <50	CAD <60	CAD <66
case	32	52	99	128
control	437	437	437	437
N	469	489	536	565

Variants included

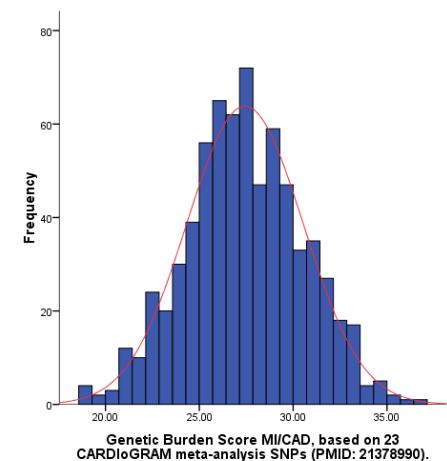
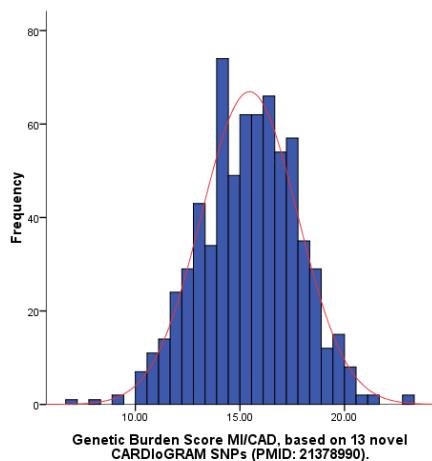
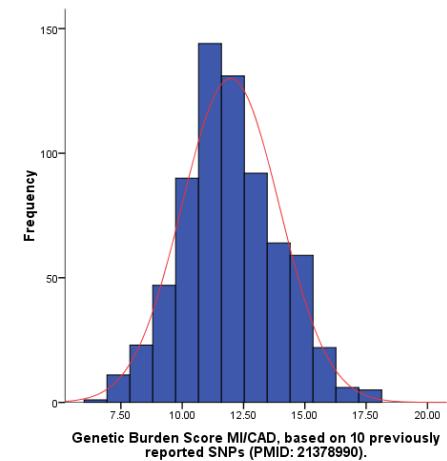
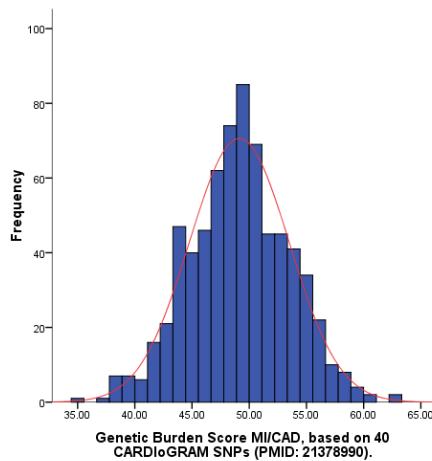
- 42 variants are “discovered”, of which 23 met the final meta-analysis criteria
- 2 variants are not in HM2r22, and therefore cannot be imputed
- 13 variants are genotyped, 27 are imputed
- 4 different *genetic burden scores* can be calculated



dbSNP rs#	Chr.	Position (bp)	Gene	Risk allele	Risk allele frequency (CARDIoGRAM)	Risk allele frequency (Athero-Express)	Odds ratio	95% CI lower upper	P-value	Analysis source	Type	Athero-Express Information			Genetic Burden Score Type				
												Source	A-allele	B-allele	Allele discovery	previous	novel	meta	
rs599839	1	109623689	SORT1	A	0.78	0.79	1.11	1.08 - 1.15	2.89E-10	meta-analysis	previously reported	genotyped	A	G	A	Y	Y	.	Y
rs11206510	1	55268627	PCKS9	T	0.82	0.82	1.08	1.05 - 1.11	9.10E-08	combined analysis	previously reported	genotyped	C	T	B	Y	Y	.	Y
rs17465637	1	n/a	MIA3	C	0.74	n/a	1.14	1.09 - 1.20	1.36E-08	subset studies	previously reported	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
rs6725887	2	20345130	VDR12	C	0.15	0.12	1.14	1.09 - 1.19	1.12E-09	meta-analysis	previously reported	genotyped	C	T	A	Y	Y	.	Y
rs2306374	3	139602642	MRSAS	C	0.18	0.18	1.12	1.07 - 1.16	3.34E-09	meta-analysis	previously reported	genotyped	C	T	A	Y	Y	.	Y
rs12526453	6	13035530	PHACTR1	C	0.67	0.65	1.10	1.06 - 1.13	1.15E-09	meta-analysis	previously reported	genotyped	C	G	A	Y	Y	.	Y
rs3798220	6	n/a	LPA	C	0.02	n/a	1.54	1.36 - 1.74	9.62E-12	replication	previously reported	n/a	n/a	n/a	n/a	n/a	n/a	n/a	
rs4977574	9	22088574	CDKN2A/B,ANRIL	G	0.46	0.48	1.29	1.23 - 1.36	1.35E-22	meta-analysis	previously reported	genotyped	A	G	B	Y	Y	.	Y
rs1746048	10	44095830	CXL12	C	0.87	0.87	1.09	1.07 - 1.13	2.12E-10	combined analysis	previously reported	genotyped	C	T	A	Y	Y	.	Y
rs3184504	12	110368991	SH2B3	T	0.44	0.53	1.07	1.04 - 1.10	6.35E-06	meta-analysis	previously reported	imputed	C	T	B	Y	Y	.	Y
rs1122608	19	11024601	LDLR	G	0.77	0.77	1.14	1.09 - 1.18	9.73E-10	meta-analysis	previously reported	genotyped	G	T	A	Y	Y	.	Y
rs9982601	21	34520998	MRPS6	T	0.15	0.12	1.18	1.12 - 1.24	4.22E-10	meta-analysis	previously reported	imputed	C	T	B	Y	Y	.	Y
rs17114036	1	56735409	PPAP2B	A	0.91	0.93	1.17	1.13 - 1.22	3.81E-19	combined analysis	new locus	imputed	A	G	A	Y	.	Y	Y
rs17609940	6	35142778	ANKS1A	G	0.75	0.80	1.07	1.05 - 1.10	1.07E-08	combined analysis	new locus	genotyped	C	G	B	Y	.	Y	Y
rs12190287	6	134256218	TCF21	C	0.62	0.66	1.08	1.06 - 1.10	1.07E-12	combined analysis	new locus	imputed	C	G	A	Y	.	Y	Y
rs11556924	7	129450732	ZC3H1C	C	0.62	0.59	1.10	1.07 - 1.12	5.68E-18	combined analysis	new locus	genotyped	C	T	A	Y	.	Y	Y
rs579459	9	135143989	ABO	C	0.21	0.23	1.10	1.07 - 1.13	4.08E-14	combined analysis	new locus	imputed	C	T	A	Y	.	Y	Y
rs12413409	10	104709086	CYP17A1,CNNM2,NT5C2	G	0.89	0.92	1.12	1.08 - 1.16	9.51E-10	combined analysis	new locus	genotyped	A	G	B	Y	.	Y	Y
rs964184	11	116154127	ZNF259,APOA5-A4-C3-A1	G	0.13	0.15	1.13	1.10 - 1.17	3.14E-18	combined analysis	new locus	imputed	C	G	B	Y	.	Y	Y
rs4773144	13	109758713	COL4A1, COL4A2	G	0.44	0.43	1.07	1.05 - 1.10	3.83E-09	combined analysis	new locus	imputed	A	G	B	Y	.	Y	Y
rs2895811	14	99203695	HH1PL1HH1PL1	C	0.43	0.45	1.07	1.05 - 1.10	4.03E-10	combined analysis	new locus	imputed	C	T	A	Y	.	Y	Y
rs3825807	15	76876166	ADAMTS7	A	0.57	0.56	1.08	1.06 - 1.10	1.07E-12	combined analysis	new locus	imputed	A	G	A	Y	.	Y	Y
rs12936587	17	17484447	RASD1,SMCR3,PEMT	G	0.56	0.52	1.07	1.05 - 1.09	4.45E-10	combined analysis	new locus	imputed	A	G	B	Y	.	Y	Y
rs216172	17	2073254	SMG6,SRR	C	0.37	0.33	1.07	1.05 - 1.09	1.18E-09	combined analysis	new locus	imputed	C	G	A	Y	.	Y	Y
rs46522	17	44343596	UBE2Z,GIP,ATP5G1,SNF8	T	0.53	0.53	1.06	1.04 - 1.08	9.03E-09	combined analysis	new locus	imputed	C	T	B	Y	.	Y	Y
rs2404715	1	56781366	-	C	0.92	0.93	1.16	1.12 - 1.20	3.75E-15	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	C	T	A	Y	.	.	.
rs10933436	2	233707625	-	A	0.49	0.46	1.06	1.04 - 1.09	7.06E-06	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	C	A	Y	.	.	.
rs7651039	3	15623008	-	C	0.54	0.53	1.06	1.04 - 1.09	1.64E-06	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	C	T	A	Y	.	.	.
rs9838412	3	86202991	-	C	0.79	0.83	1.04	1.01 - 1.08	7.50E-03	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	C	B	Y	.	.	.
rs12190423	6	72259432	-	G	0.61	0.64	1.05	1.02 - 1.07	5.42E-05	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	C	G	B	Y	.	.	.
rs12524865	6	134238367	-	C	0.70	0.73	1.07	1.04 - 1.09	2.29E-08	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	C	B	Y	.	.	.
rs7808424	7	116855058	-	G	0.12	0.08	1.10	1.06 - 1.14	1.17E-06	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	G	T	A	Y	.	.	.
rs12682131	8	137311147	-	G	0.93	0.99	1.10	1.02 - 1.19	1.05E-02	combined analysis	new locus, not meeting CARDIoGRAM criteria	genotyped	A	G	B	Y	.	.	.
rs651007	9	135143696	-	T	0.19	0.23	1.10	1.07 - 1.13	2.36E-13	combined analysis	new locus, not meeting CARDIoGRAM criteria	genotyped	C	T	B	Y	.	.	.
rs7920682	10	30357832	-	A	0.54	0.59	1.05	1.02 - 1.08	1.44E-04	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	G	A	Y	.	.	.
rs16911227	10	59369844	-	G	0.93	0.96	1.09	1.03 - 1.15	2.31E-03	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	G	B	Y	.	.	.
rs12411886	10	104675289	-	C	0.89	0.92	1.11	1.07 - 1.15	5.59E-09	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	C	B	Y	.	.	.
rs4937126	11	125787107	-	G	0.69	0.71	1.06	1.04 - 1.09	4.73E-06	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	G	B	Y	.	.	.
rs4624107	14	99197193	-	C	0.44	0.45	1.07	1.05 - 1.09	6.75E-09	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	C	G	A	Y	.	.	.
rs12924776	16	88114093	-	T	0.20	0.20	1.06	1.03 - 1.08	9.93E-06	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	T	B	Y	.	.	.
rs1231206	17	2072355	-	A	0.37	0.33	1.07	1.05 - 1.09	8.52E-10	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	A	G	A	Y	.	.	.
rs12449964	17	17485429	-	C	0.56	0.52	1.06	1.04 - 1.09	8.43E-09	combined analysis	new locus, not meeting CARDIoGRAM criteria	imputed	C	T	A	Y	.	.	.

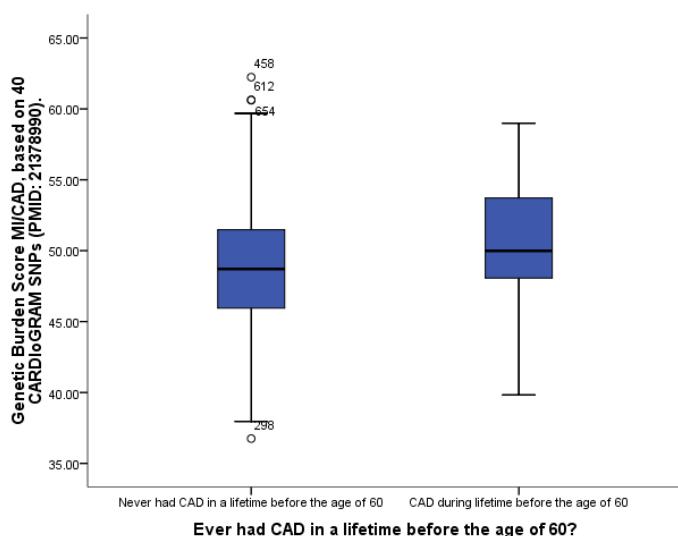
Genetic Burden Scores of CAD variants

- 4 GBS' are constructed, including:
 - 40 SNPs associated with CAD in *discovery* phase
 - 10 *previously reported* SNPs associated with CAD
 - 13 *novel* reported SNPs associated with CAD
 - 23 SNPs *combined genome-wide significantly associated* with CAD



CAD <60 years per GBS category

discovery phase & previously associated



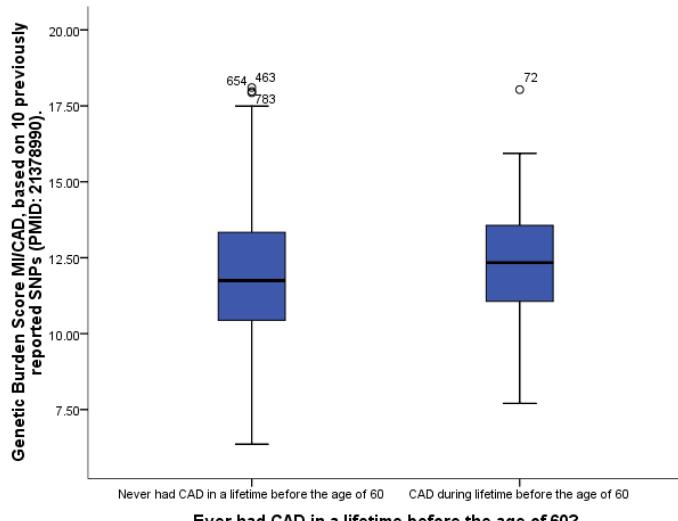
Ranks		
Genetic Burden Score MI/CAD, based on 40 CARDIoGRAM SNPs (PMID: 21378990).	N	Mean Rank
Ever had CAD in a lifetime before the age of 60	437	257.13
Never had CAD in a lifetime before the age of 60	99	318.70
Total	536	

Test Statistics ^{b,c}		
Genetic Burden Score MI/CAD, based on 40 CARDIoGRAM SNPs (PMID: 21378990).		
Chi-Square	12.756	
df	1	
Asymp. Sig.	.355E-4	
Monte Carlo Sig.	.001 ^a	
99% Confidence Interval	Lower Bound	.000
	Upper Bound	.001

a. Based on 10000 sampled tables with starting seed 1502173562.

b. Kruskal Wallis Test

c. Grouping Variable: Ever had CAD in a lifetime before the age of 60?



Ranks		
Genetic Burden Score MI/CAD, based on 10 previously reported SNPs (PMID: 21378990).	N	Mean Rank
Ever had CAD in a lifetime before the age of 60	437	261.40
Never had CAD in a lifetime before the age of 60	99	299.83
Total	536	

Test Statistics ^{b,c}		
Genetic Burden Score MI/CAD, based on 10 previously reported SNPs (PMID: 21378990).		
Chi-Square	4.969	
df	1	
Asymp. Sig.	.026	
Monte Carlo Sig.	.026 ^a	
99% Confidence Interval	Lower Bound	.022
	Upper Bound	.030

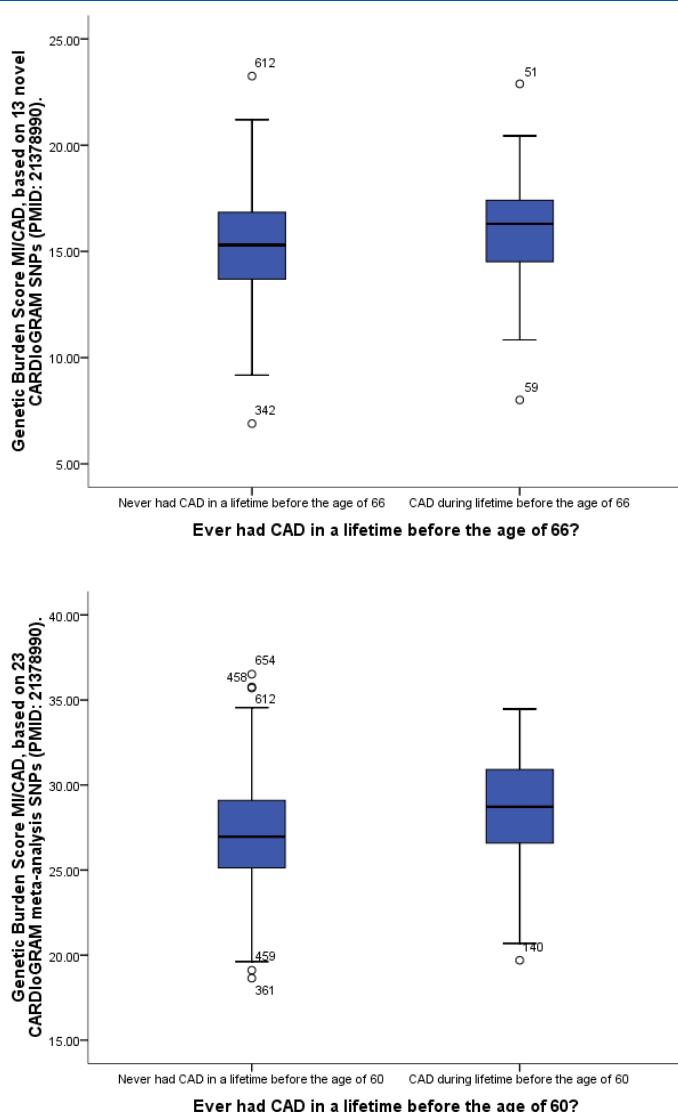
a. Based on 10000 sampled tables with starting seed 562334227.

b. Kruskal Wallis Test

c. Grouping Variable: Ever had CAD in a lifetime before the age of 60?

CAD <60 years per GBS category

novel variants & final meta-analysis



Ranks

Ever had CAD in a lifetime before the age of 60?	N	Mean Rank
Never had CAD in a lifetime before the age of 60	437	254.98
CAD during lifetime before the age of 60	99	328.16
Total	536	261.57

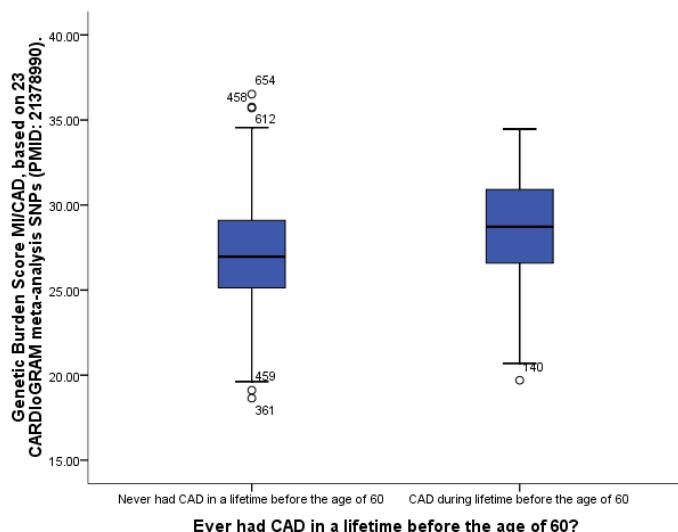
Test Statistics^{b,c}

	Genetic Burden Score MiICAD, based on 13 novel CARDioGRAM SNPs (PMID: 21378990)
Chi-Square	18.020
df	1
Asymp. Sig.	2.19E-5
Monte Carlo Sig.	.000 ^a
Sig.	.000
99% Confidence Interval	Lower Bound .000 Upper Bound .000

a. Based on 10000 sampled tables with starting seed 1585587178.

b. Kruskal Wallis Test

c. Grouping Variable: Ever had CAD in a lifetime before the age of 60?



Ranks

Ever had CAD in a lifetime before the age of 60?	N	Mean Rank
Never had CAD in a lifetime before the age of 60	361	253.46
CAD during lifetime before the age of 60	654	334.88
Total	536	294.17

Test Statistics^{b,c}

	Genetic Burden Score MiICAD, based on 23 CARDioGRAM meta-analysis SNPs (PMID: 21378990)
Chi-Square	22.306
df	1
Asymp. Sig.	2.32E-6
Monte Carlo Sig.	.000 ^a
Sig.	.000
99% Confidence Interval	Lower Bound .000 Upper Bound .000

a. Based on 10000 sampled tables with starting seed 1122541128.

b. Kruskal Wallis Test

c. Grouping Variable: Ever had CAD in a lifetime before the age of 60?

Does age really matter?

Comparison medians of Genetic Burden Scores for Coronary Artery Disease

Age group	N cases	N controls	GBS type	cases			controls			P -value
				median	lower percentile	higher percentile	median	lower percentile	higher percentile	
<45 years	32	437	discovery	49.44	47.09	54.61	48.71	45.95	51.48	1.68E-01
			previous	12.43	10.70	14.24	11.75	10.44	13.34	2.82E-01
			novel	15.84	14.69	17.20	15.30	13.69	16.84	1.54E-01
			meta-analysis	28.01	26.18	30.49	26.96	25.13	29.09	4.95E-02
<50 years	52	437	discovery	49.52	48.13	53.85	11.75	45.95	51.48	1.28E-02
			previous	12.14	10.43	13.44	11.75	10.44	13.34	3.56E-01
			novel	16.38	15.35	17.38	11.75	13.69	16.84	1.62E-03
			meta-analysis	28.38	26.36	30.17	11.75	25.13	29.09	1.86E-03
<60 years	99	437	discovery	49.99	48.06	53.72	15.30	45.95	51.48	3.55E-04
			previous	12.34	11.07	13.56	15.30	10.44	13.34	2.58E-02
			novel	16.36	15.08	17.51	15.30	13.69	16.84	2.19E-05
			meta-analysis	28.72	26.59	30.92	15.30	25.13	29.09	2.32E-06
<66 years	128	437	discovery	49.55	47.59	53.68	26.96	45.95	51.48	1.85E-03
			previous	12.31	11.10	13.48	26.96	10.44	13.34	2.31E-02
			novel	16.29	14.51	17.41	26.96	13.69	16.84	6.78E-05
			meta-analysis	28.50	26.43	30.81	26.96	25.13	29.09	6.23E-06

Logistic regression for CAD age groups

- Covariates: age at onset for cases, age at inclusion for controls, gender
 - The novel variants and the final meta-analysis variants are best in predicting CAD < 60-66 years
 - It appears that these variants do not predict CAD before 50 years...

GBS type	Age group	Effect size (β)	SE β	Odds ratio	95% CI		P -value
					lower	upper	
discovery	< 45 years	-0.100	0.083	0.905	0.768	1.065	0.230
	< 50 years	-0.053	0.071	0.948	0.826	1.089	0.449
	< 60 years	0.047	0.038	1.048	0.973	1.130	0.216
	< 66 years	0.034	0.030	1.034	0.976	1.097	0.257
previous	< 45 years	0.022	0.171	1.022	0.732	1.428	0.896
	< 50 years	-0.128	0.137	0.880	0.673	1.151	0.351
	< 60 years	0.077	0.079	1.080	0.926	1.260	0.327
	< 66 years	0.058	0.064	1.059	0.934	1.201	0.368
novel	< 45 years	-0.213	0.180	0.808	0.567	1.151	0.238
	< 50 years	0.123	0.134	0.880	0.673	1.151	0.360
	< 60 years	0.166	0.075	1.181	1.020	1.368	0.026
	< 66 years	0.126	0.058	1.134	1.013	1.270	0.029
meta-analysis	< 45 years	-0.070	0.110	0.932	0.752	1.156	0.523
	< 50 years	-0.002	0.092	0.998	0.833	1.196	0.981
	< 60 years	0.126	0.054	1.134	1.019	1.262	0.021
	< 66 years	0.096	0.043	1.100	1.012	1.197	0.026



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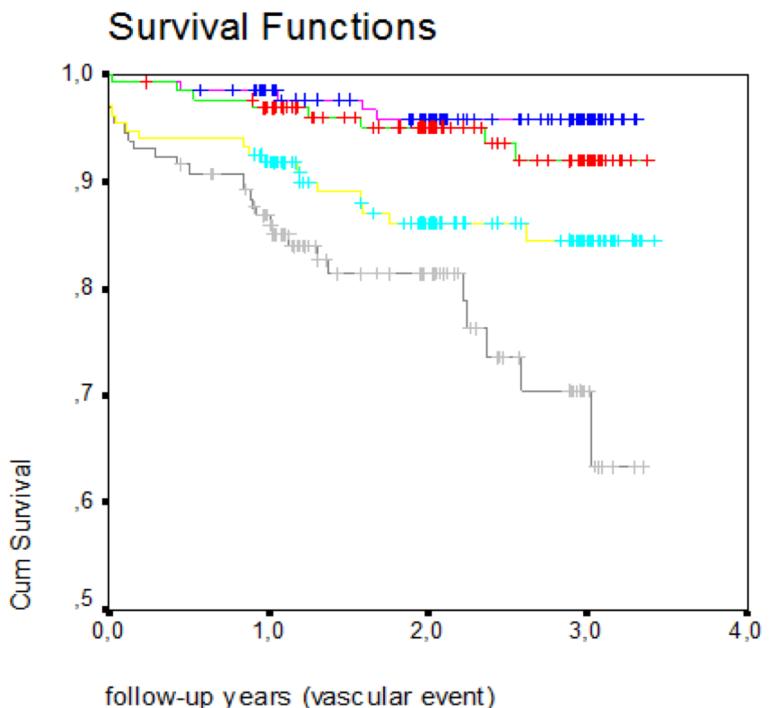
Genetics, Biomarkers & Disease

OPN AS A BIOMARKER



Some background information

- Osteopontin (OPN) expression in plaques is predictive for future vascular events
- Is OPN a good biomarker?



Local Atherosclerotic Plaques Are a Source of Prognostic Biomarkers for Adverse Cardiovascular Events

Dominique P.V. de Kleijn, Frans L. Moll, Willem E. Hellings, Gonen Ozsarlak-Sozer, Peter de Bruin, Pieter A. Doevedans, Aryan Vink, Louise M. Catanzariti, Arjan H. Schoneveld, Ale Algra, Mat J. Daemen, E.A. Biessen, W. de Jager, Huoming Zhang, Jean-Paul de Vries, Erling Falk, Sai K. Lim, Peter J. van der Spek, Siu Kwan Sze, Gerard Pasterkamp

Objective—Atherosclerotic cardiovascular disease is a major burden to health care. Because atherosclerosis is considered a systemic disease, we hypothesized that one single atherosclerotic plaque contains ample molecular information that predicts future cardiovascular events in all vascular territories.

Methods and Results—AtheroExpress is a biobank collecting atherosclerotic lesions during surgery, with a 3-year follow-up. The composite primary outcome encompasses all cardiovascular events and interventions, eg, cardiovascular death, myocardial infarction, stroke, and endovascular interventions. A proteomics search identified osteopontin as a potential plaque biomarker. Patients undergoing carotid surgery ($n=574$) served as the cohort in which plaque osteopontin levels were examined in relation to their outcome during follow-up and was validated in a cohort of patients undergoing femoral endarterectomy ($n=151$). Comparing the highest quartile of carotid plaque osteopontin levels with quartile 1 showed a hazard ratio for the primary outcome of 3.8 (95% confidence interval, 2.6–5.9). The outcome did not change after adjustment for plaque characteristics and traditional risk factors (hazard ratio, 3.5; 95% confidence interval, 2.0–5.9). The femoral validation cohort showed a hazard ratio of 3.8 (95% confidence interval 2.0 to 7.4) comparing osteopontin levels in quartile 4 with quartile 1.

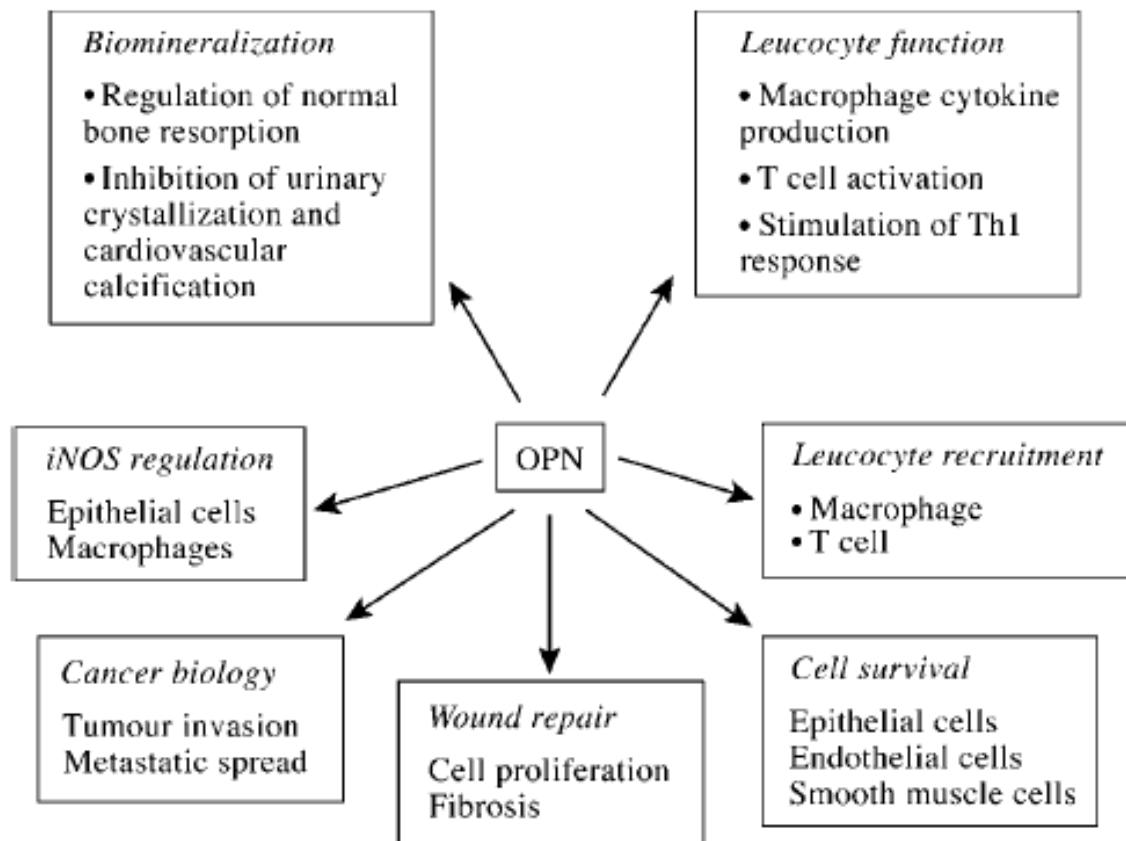
Conclusion—Plaque osteopontin levels in single lesions are predictive for cardiovascular events in other vascular territories. Local atherosclerotic plaques are a source of prognostic biomarkers with a high predictive value for secondary manifestations of atherosclerotic disease. (*Arterioscler Thromb Vasc Biol*. 2010;30:612–619).

Key Words: arterectomy ■ atherosclerosis ■ biomarker ■ plaque

More on OPN

- What constitutes a good biomarker?

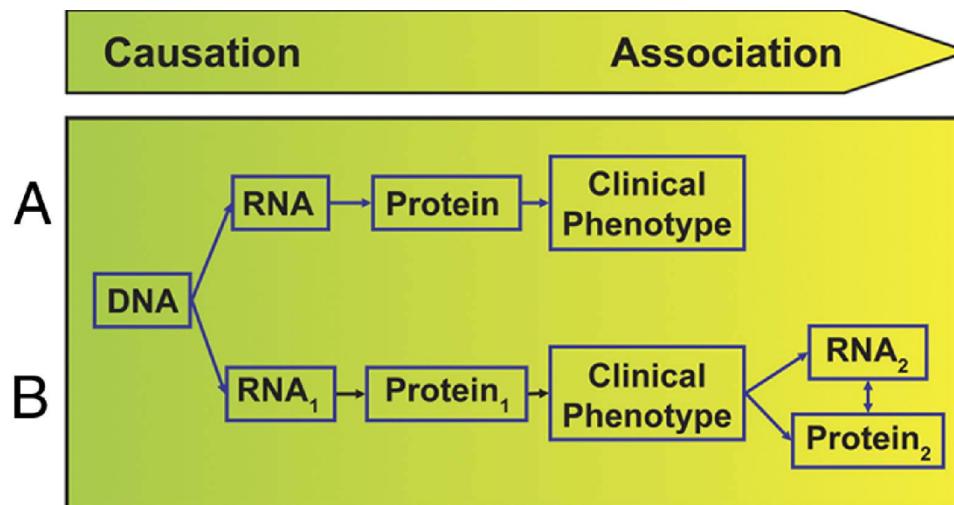
- Mechanistically involved
- Preferably causal
- Measurable
- Treatable



Biomarkers & Disease

chicken or the egg

- A biomarker can be a protein causing the disease
- A biomarker can be a protein associated with disease



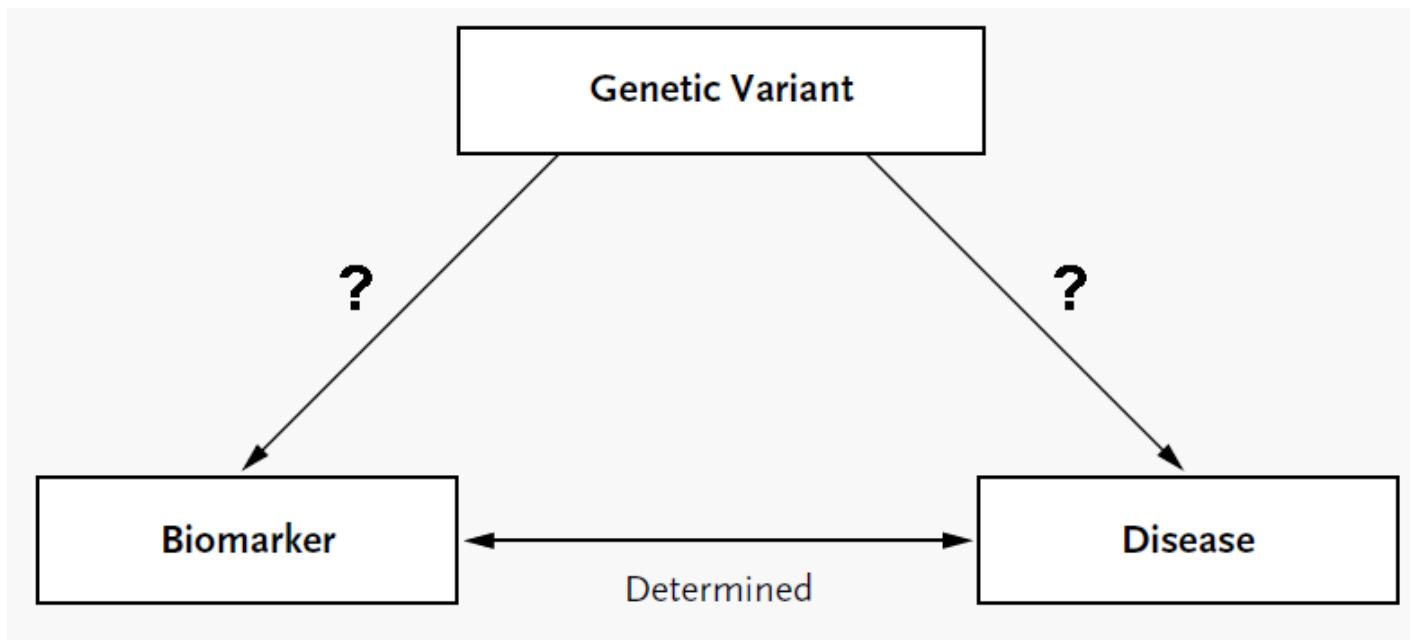
SNPs, Biomarkers & Disease

can genetic variation tell us something?



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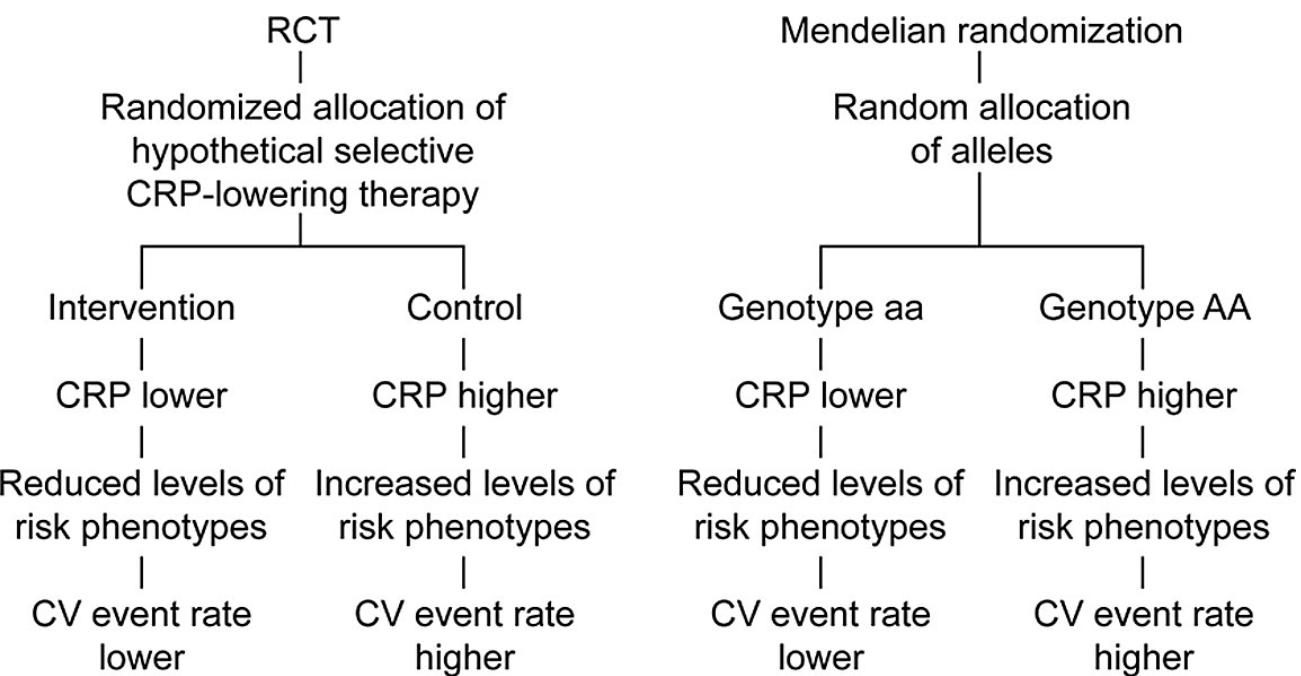
- A biomarker is associated with disease
- A genetic variant is associated with the expression of the biomarker
- A genetic variants is associated with disease



Mendelian Randomization

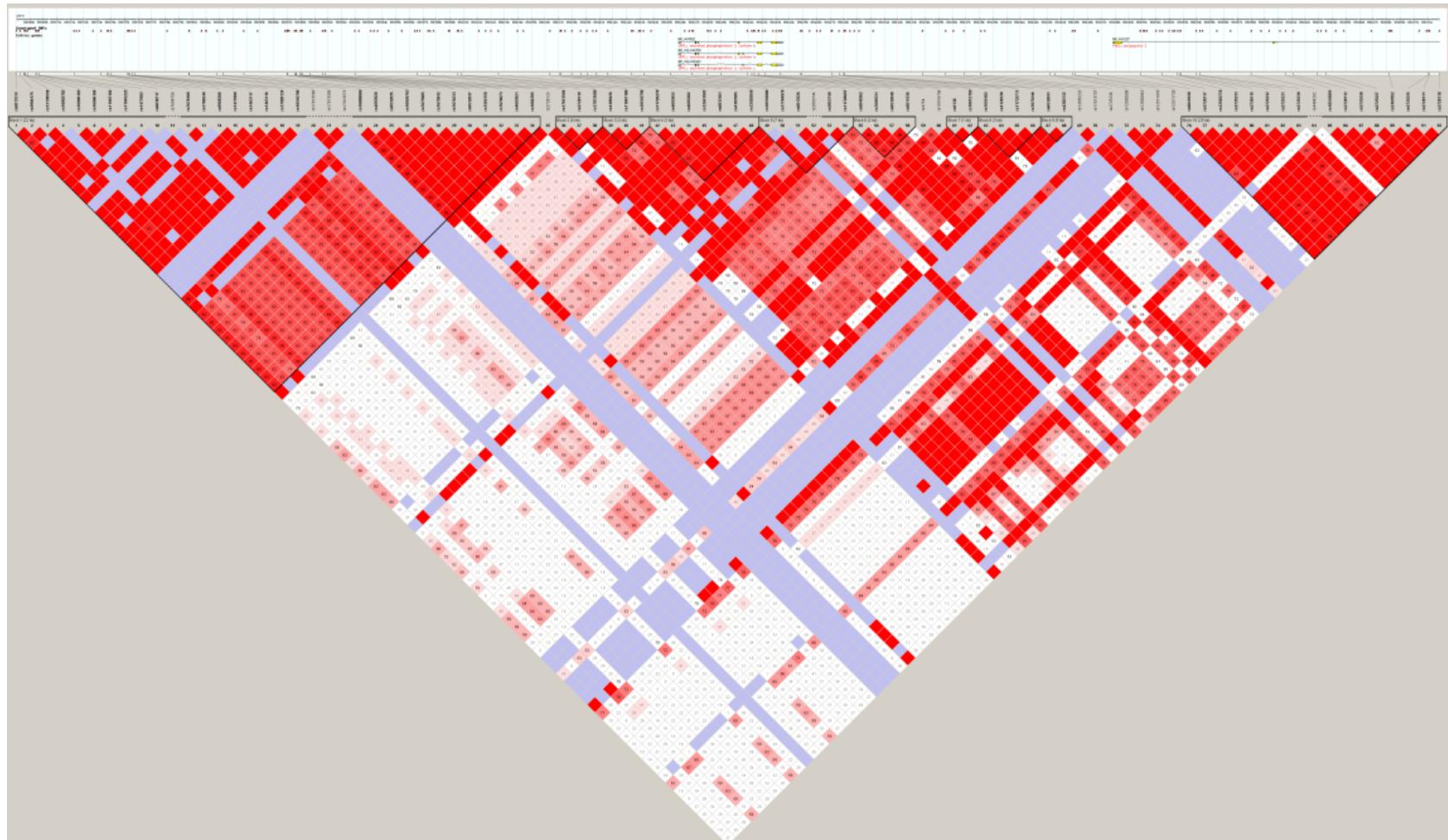
can genetic variation tell us something?

- We can't imagine a randomized double-blind clinical trial with human subjects while knocking out some of their genes... right?
- Mendelian Randomization
 - We get one copy DNA from our mother and one from our father
 - Add a little recombination in the mix



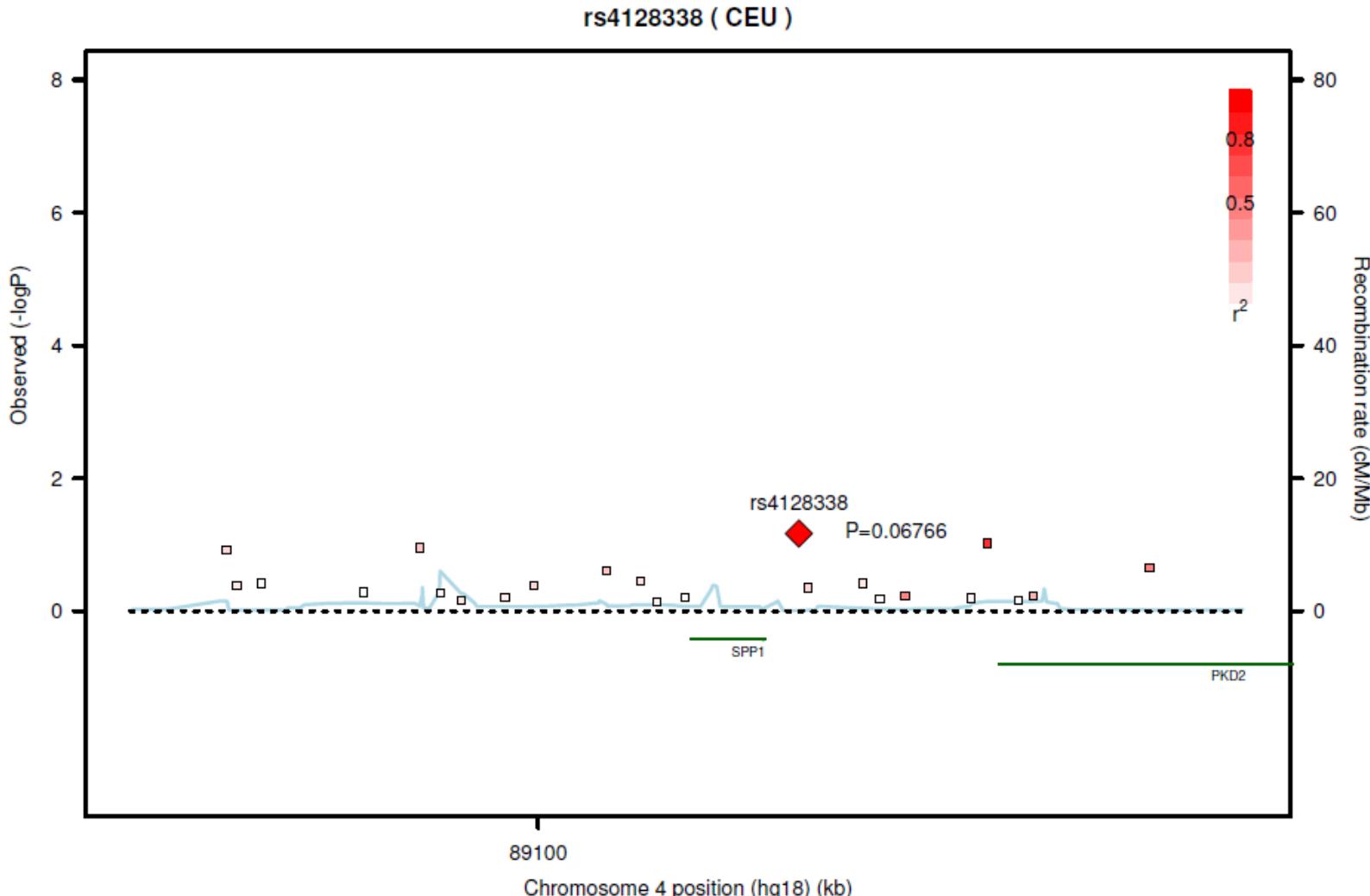
SNPs tagging OPN

- Look at LD-plot



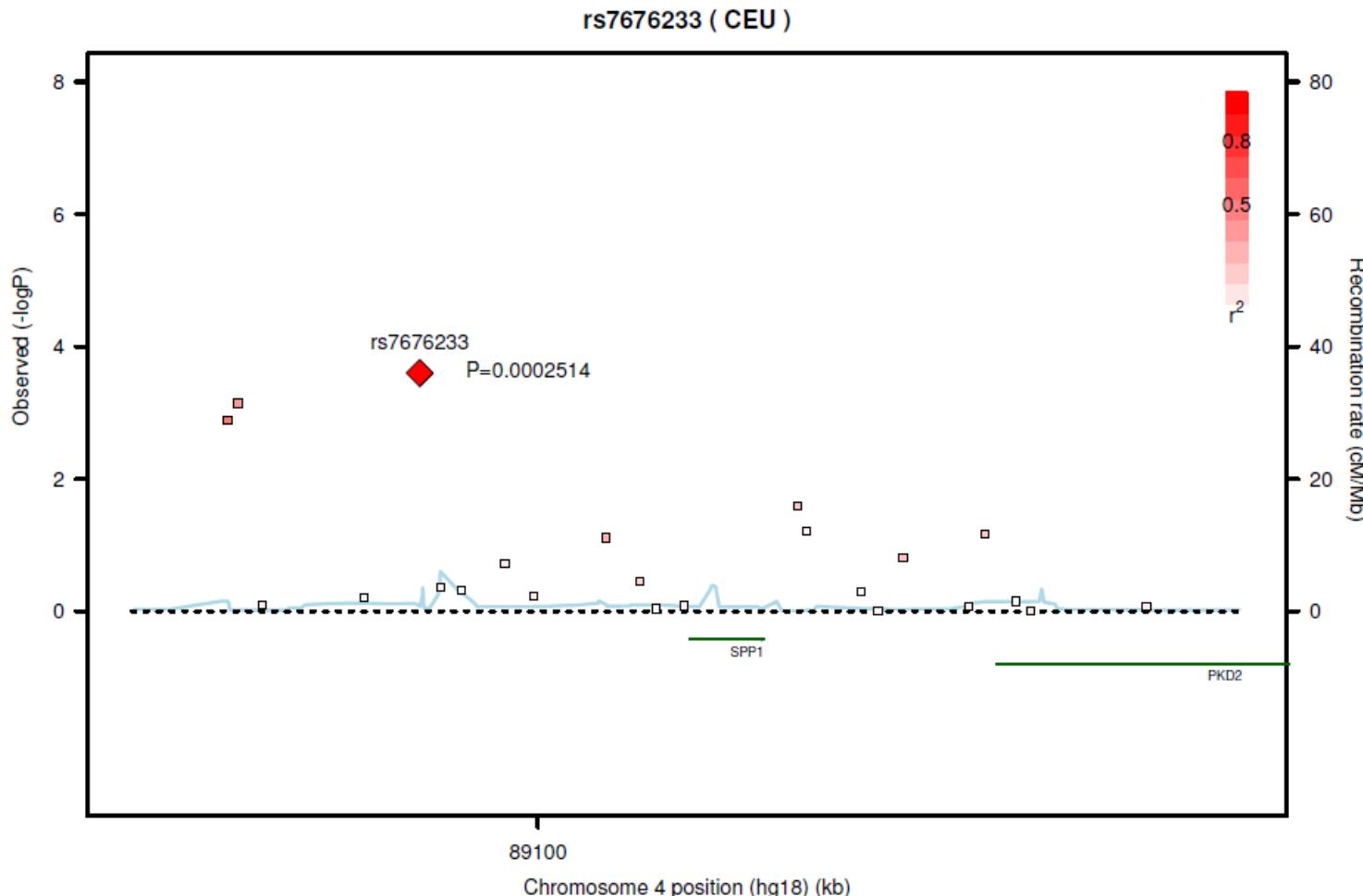
SNPs associated with OPN plaque expression

- Associate each tagSNP with the expression of OPN in the plaque



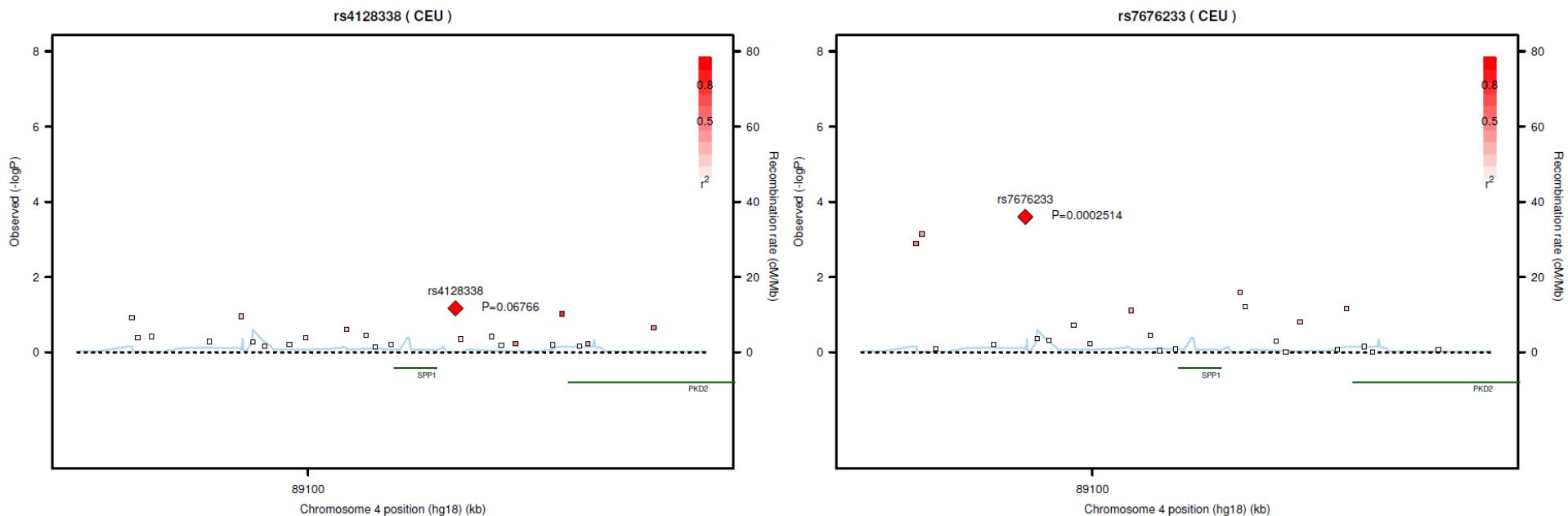
SNPs associated with OPN plasma expression

- Associate each tagSNP with the expression of OPN in the plasma



OPN & SNPs: what's next?

- Associate significant SNPs with disease
- One problem:
 - Plaque expression was associated with disease
 - Plasma expression was *not* associated!



Conclusions

- A GWAS of plaque phenotypes
 - Might identify genes involved in atherosclerotic plaque formation, development and progression
 - Explain association of vessel density with disease
- A genetic burden score
 - Could stratify patients in high and low risk groups
- Mendelian Randomization can be used
 - To understand the association of biomarkers with disease
 - Assess the causality of a biomarker

Cardiovascular Genetic Research

Experimental Cardiology Laboratory

Prof. Dr. G. Pasterkamp

Prof. Dr. D.P.V. de Kleijn

Dr. J.P.G. Sluijter

Dr. I. Höfer

Medical Genetics

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

Dr. F.W. Asselbergs

Research topics

Biomarker Discovery & Validation

Athero-Express | CTMM: Circulating Cells | Toll Express

Regenerative Medicine in Ischaemic Heart Disease

Stem Cells | Progenitor Cells

Mechanisms of Arterial Occlusive Disease

*Toll-like receptors | Regenerative Medicine |
Arteriogenesis*

Cardiovascular Genomics

*Next-Generation Sequencing | GWAS |
Pharmacogenomics*



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