

Genomics of Atherosclerosis & Cardiovascular Disease

A primer in genetics of cardiovascular disease

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What we'll discuss today...

- Recapture Some Basic Genetics
 - Human Genome & Genetic Variation
- Discoveries in Mendelian Diseases & Complex Diseases
 - Huntington's Disease
 - GWAS & Statistics
 - Coronary artery disease
 - Other examples





Recapturing Some Basic Genetics

THE HUMAN GENOME



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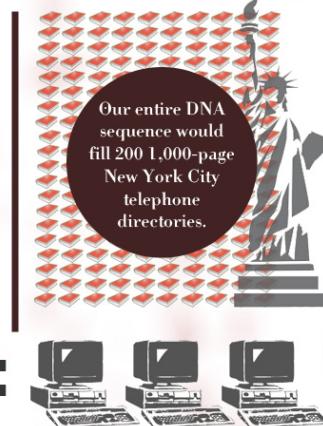
Human Genome: *some statistics*

- 3.2 billion base pairs in the haploid genome
- $\approx 18,000\text{-}25,000$ 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 and other variations
 - Transposable elements
- (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.**



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



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



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.

ATGCCGATCGTACGACACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCATCGTACTGACTGCATCGATCC
TACTGACTGCATCGTACTGACTGCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCCACATATCGTTAC
CATCGTACTGACTGTCTAGTCTAAACACATCCCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCCAGCA
CATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCTATGCCGATCGTACGACACATATCGTCATCGTACTGCC
ACTGTCTAGTCTAAACACATCCATCGTACTGACTGCATCGTACTGACTGCATCGTACTGACTGCACATATCGTCATACA
TCGTACTGACTGTCTAGTCTAAACACATCCCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCCACTTA
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CTGCTAGTCTAAACACATCCATCGTACTGACTGCATCGTACTGACTGCATCGTACTGACTGCACATATCGTCATACAT
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GTACTGACTGTCTAGTCTAAACACATCCACATATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCCACTTACC
ATCGTCATCGTACTGACTGTCTAGTCTAAACACATCCACACTGTCTAGTCTAAACACATCCATCGTACTGACTGCATC
CGATCGTACGACACATATCGTCATCGTACTGCCCTACGGGACTGTCTAGTCTAAACACATCCATCGTACTGACTGCAT

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

Human genome: *individual variations*

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

articles

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

The International SNP Map Working Group*

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

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



The International HapMap Project

Phase I

1.1 million SNPs

270 individuals from 4 populations



Phase II

3.1 million SNPs

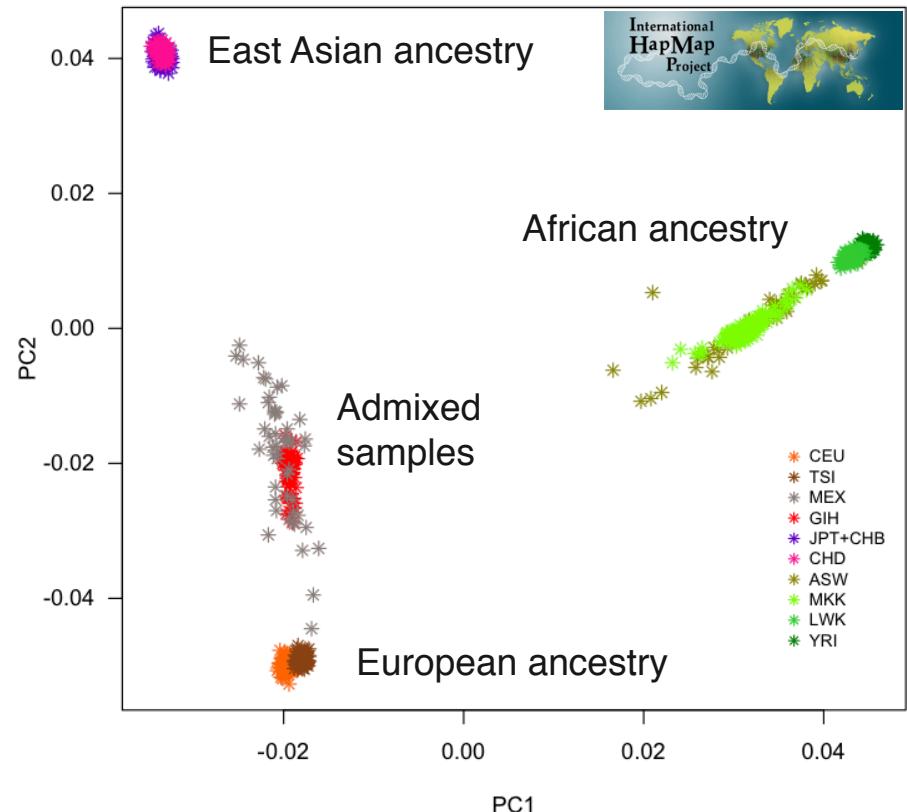
270 individuals from 4 populations



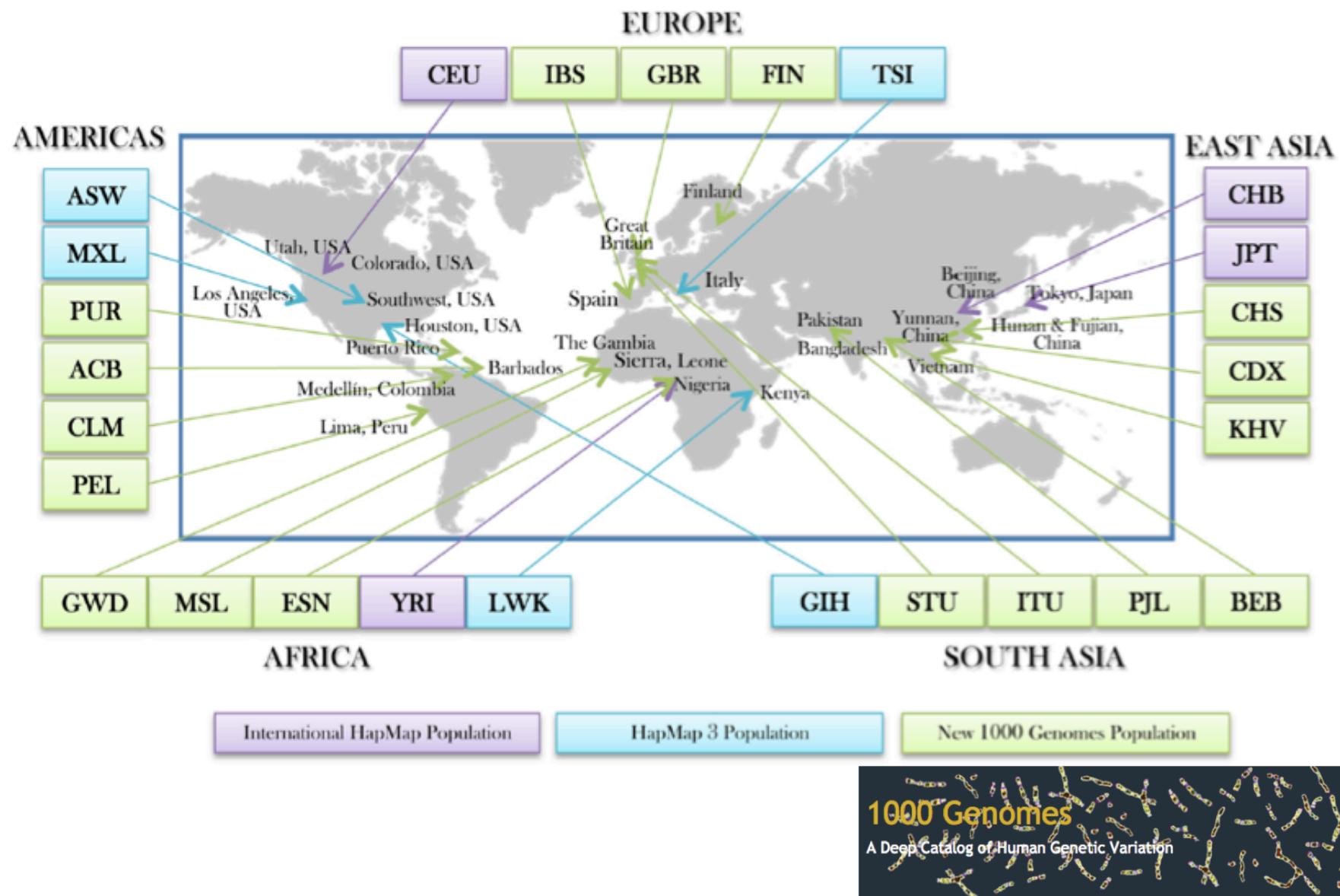
Phase III

1.6 million SNPs

1,184 individuals from 11 populations



The 1000 Genomes Project

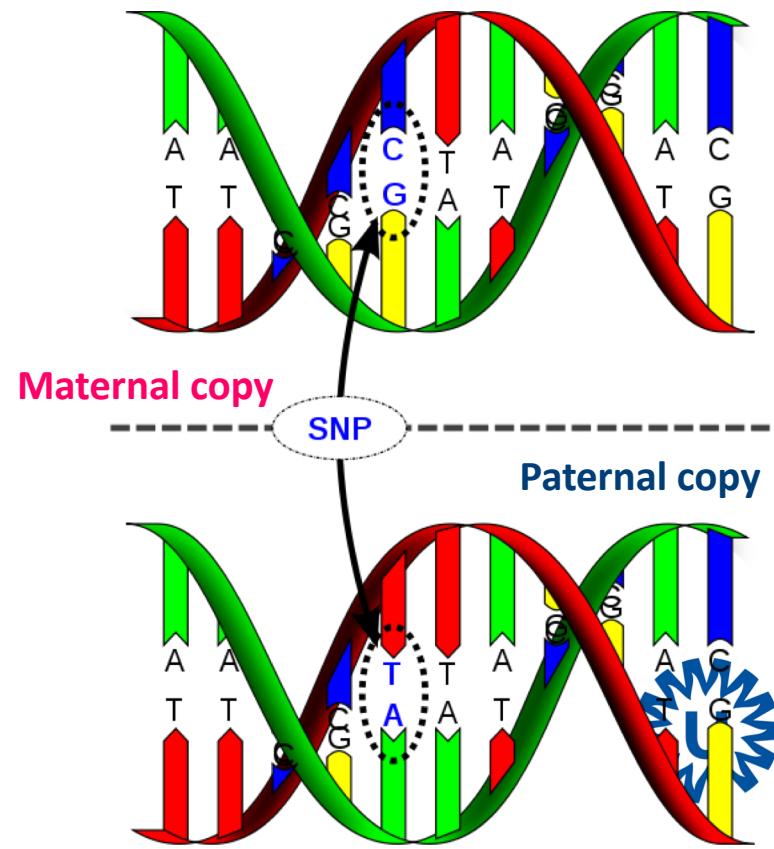


Single-Nucleotide Polymorphism

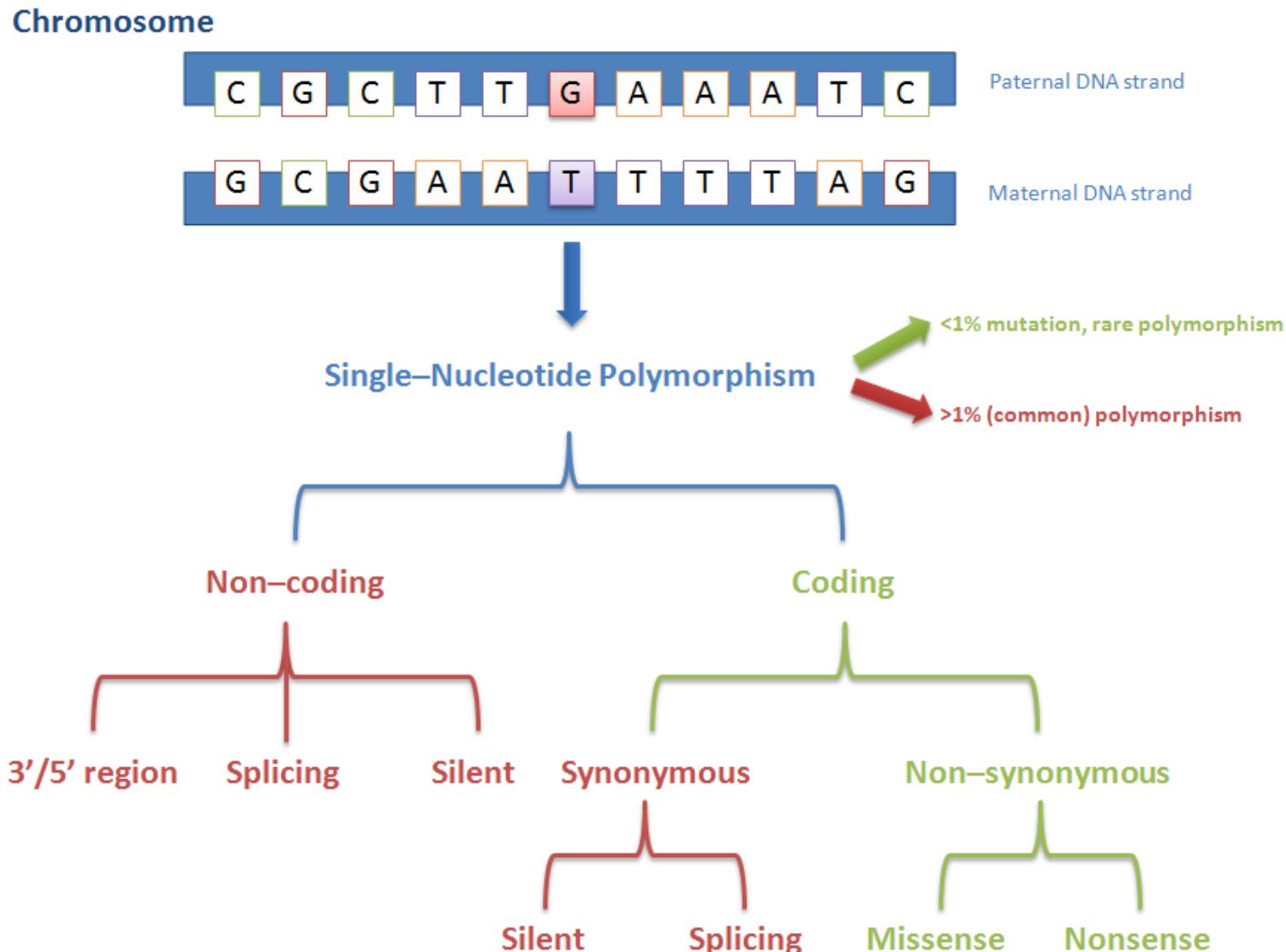
- “one base pair variation”
 - > 1% general population (common)
 - ≈10 million SNPs ($\approx 0.25\%$ genome)
 - Makes you and me unique
 - Most common type of genetic variation
- Can alter amino acid sequence
- Differential correlation between SNPs in various populations
- Used as proxies in genetic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

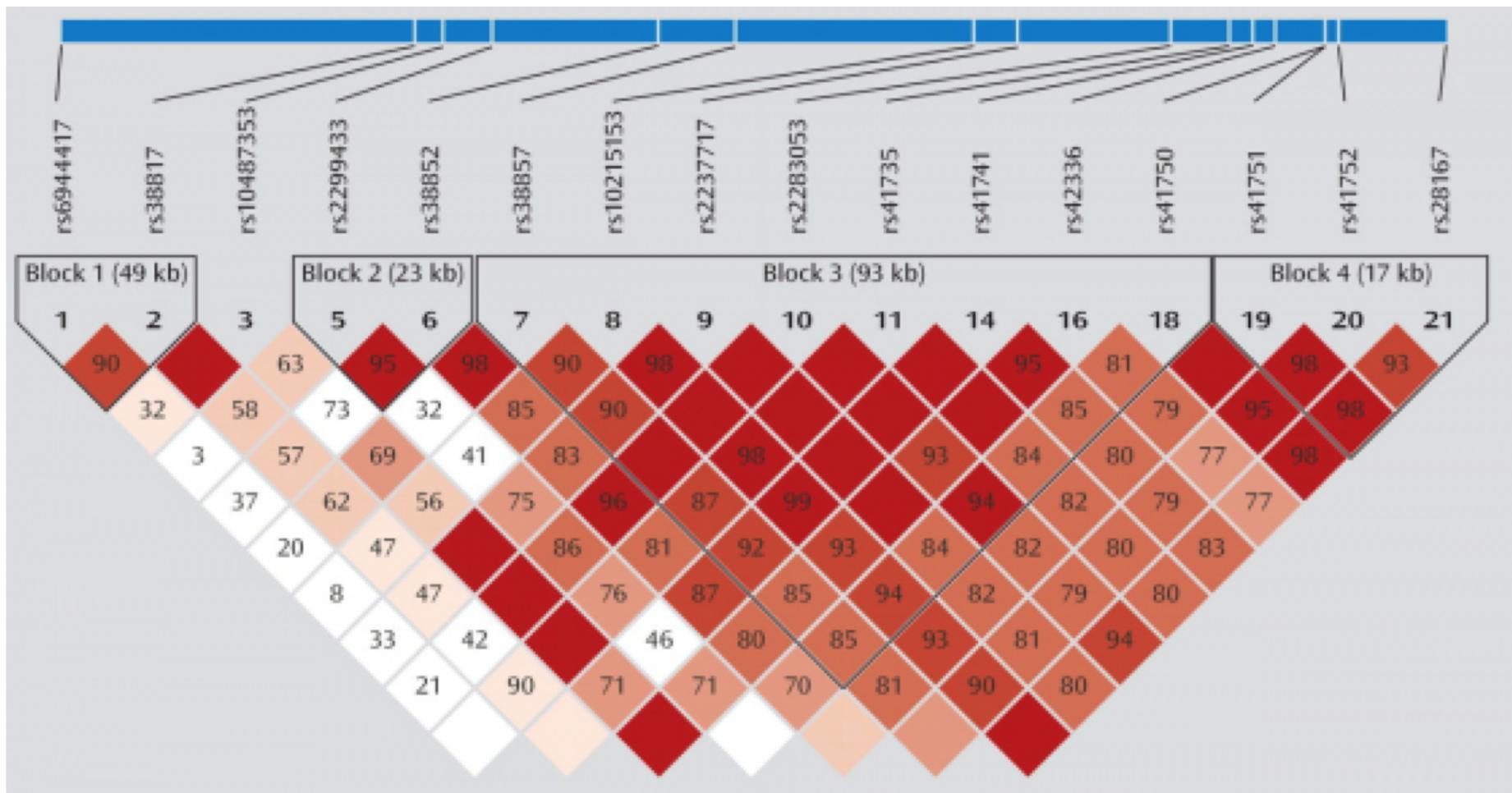


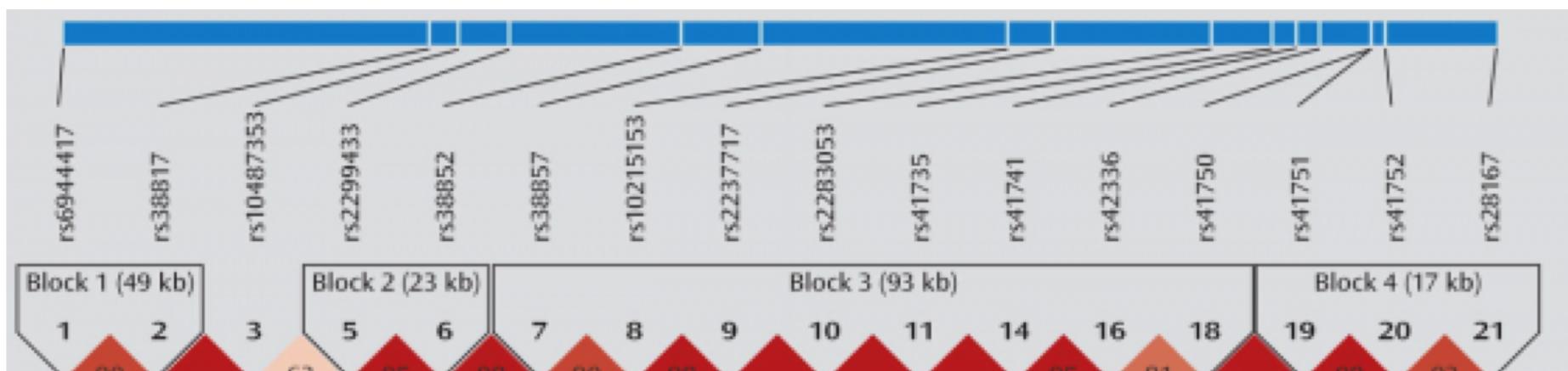
www.hapmap.org



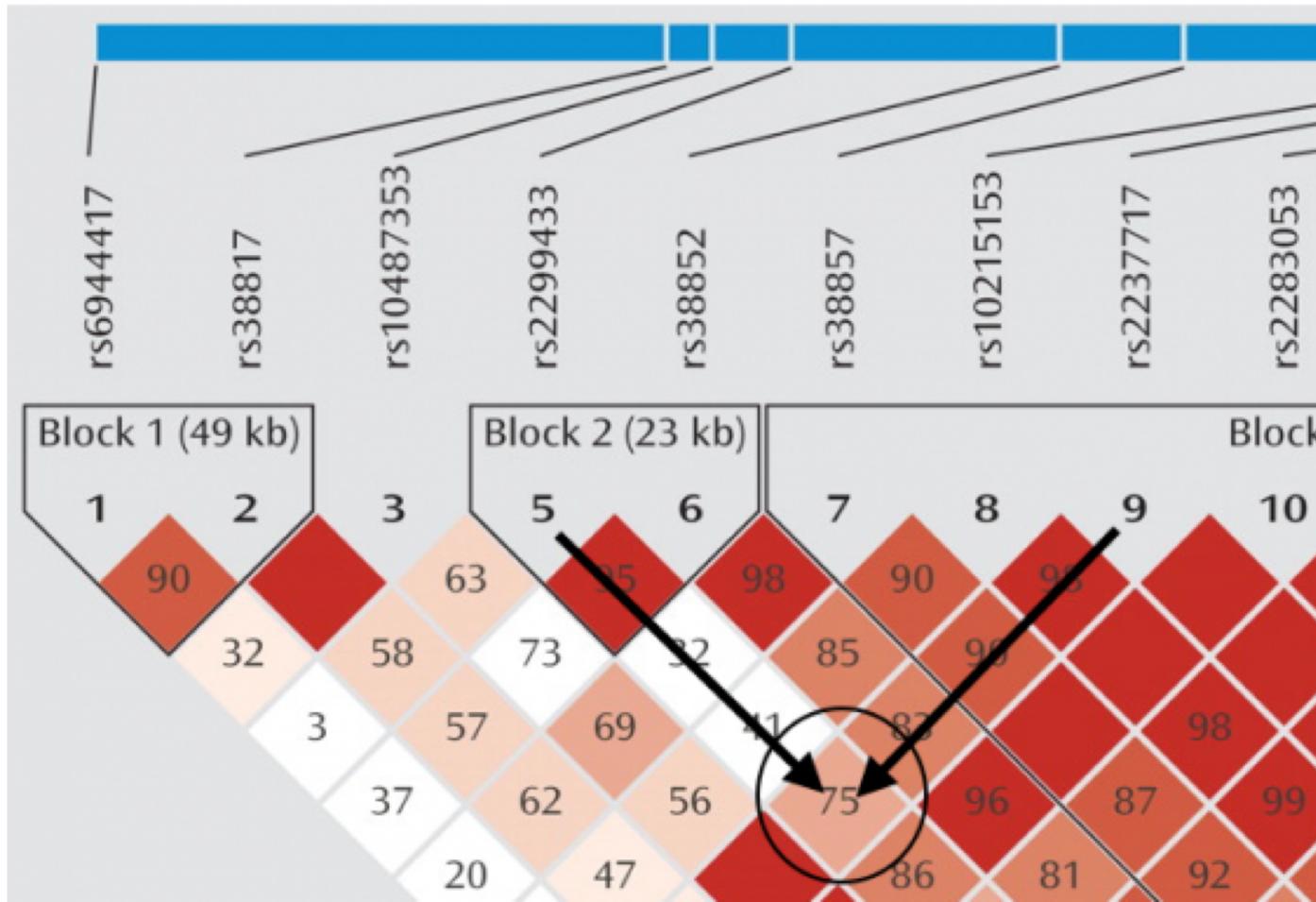
Types of SNPs

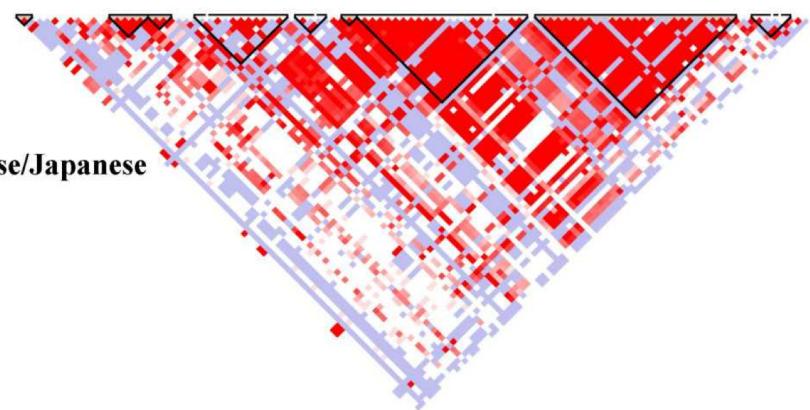
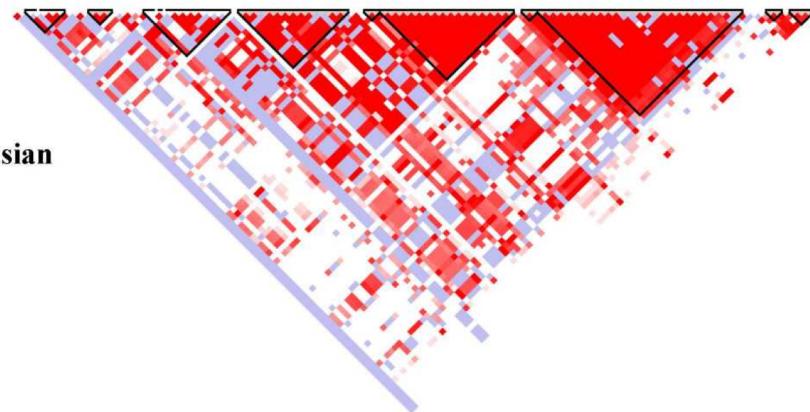






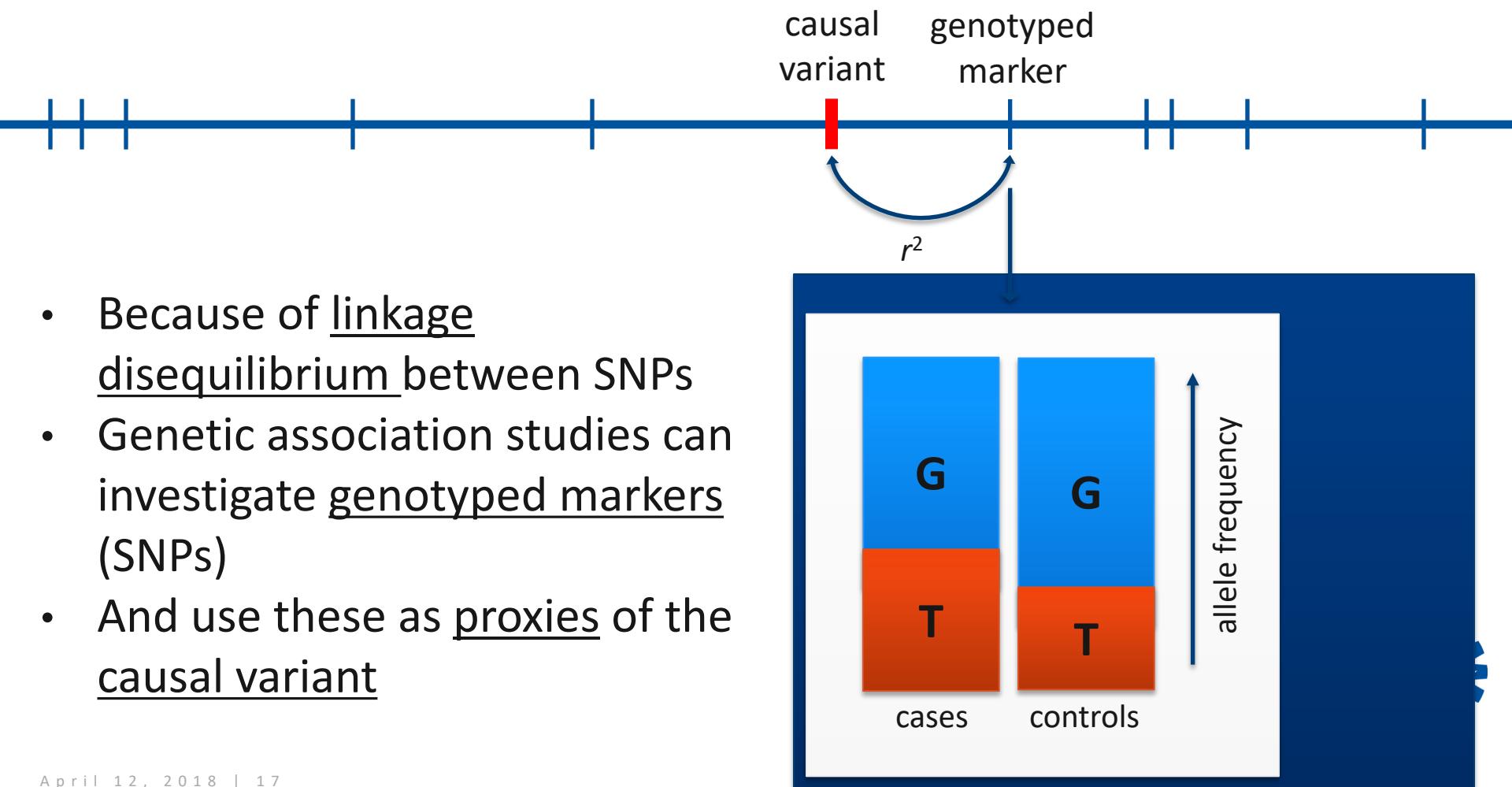






Linkage disequilibrium

Non-random association of alleles at two or more loci





3 THINGS YOU SHOULD KNOW NOW



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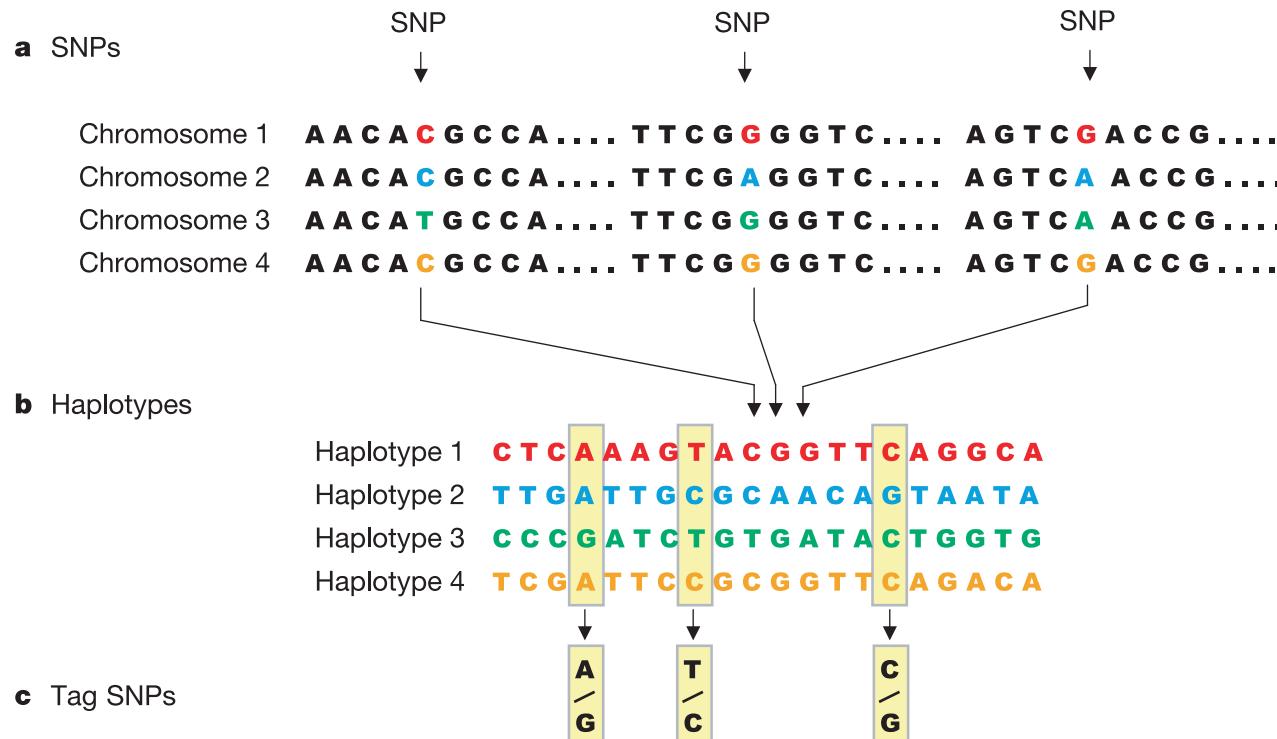
We are all equally unique

~100 million genetic variations are known to date

among ~3 billion base pairs in the human genome



These genetic variants, i.e. single-nucleotide polymorphisms (SNPs), are correlated and form *haplotypes*



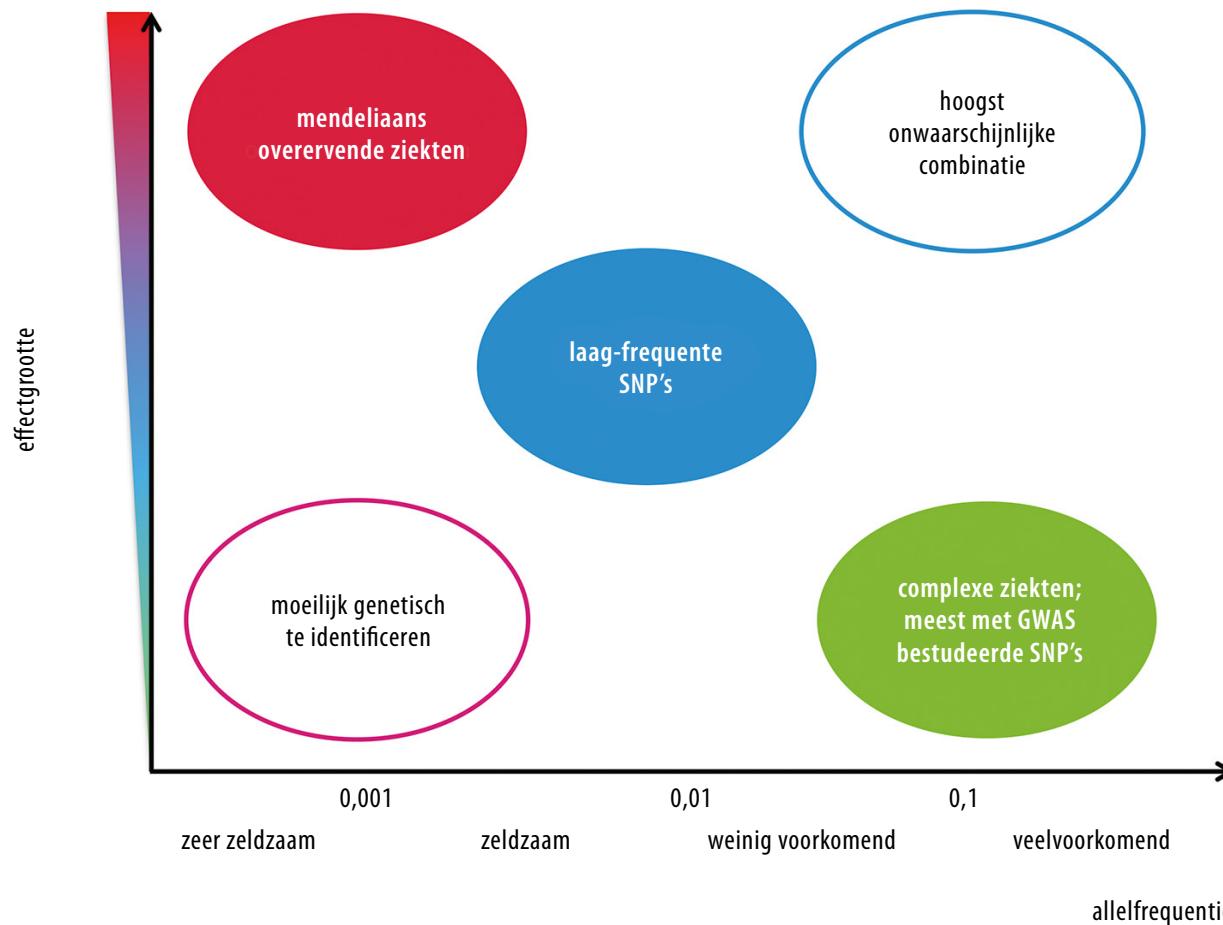
~ 1 million uncorrelated variants – thus $p < 5 \times 10^{-8}$

The International HapMap Project. *Nature*; 426:789-796; 2003

Pe'er I. *Genet Epidemiol*; 32(4):381-385; 2008

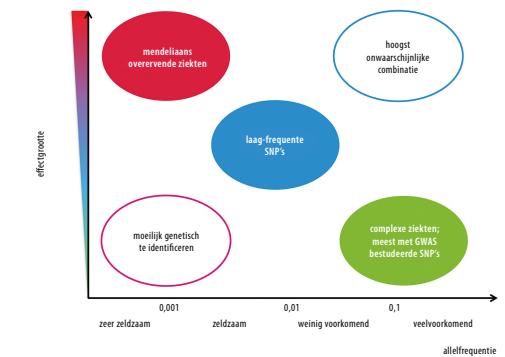
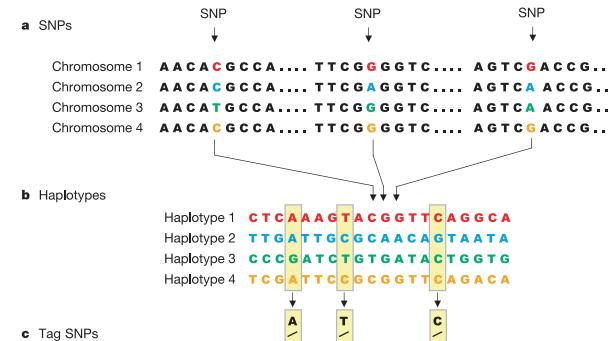
Dudbridge F. *Genet Epidemiol*; 32(3):227-234; 2008

Common diseases and traits are complex and polygenic by nature many SNPs are involved with small effects



So now you know 3 things

1. We are all equally unique: ~100 million genetic variations are known to date, among ~3 billion base pairs in the human genome
2. These genetic variants, i.e. single-nucleotide polymorphisms (SNPs), are correlated and form *haplotypes*
3. Common diseases and traits are complex and polygenic by nature: many SNPs are involved with small effects





Recapturing Some Basic Genetics

MENDELIAN AND COMPLEX DISEASES



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Family history

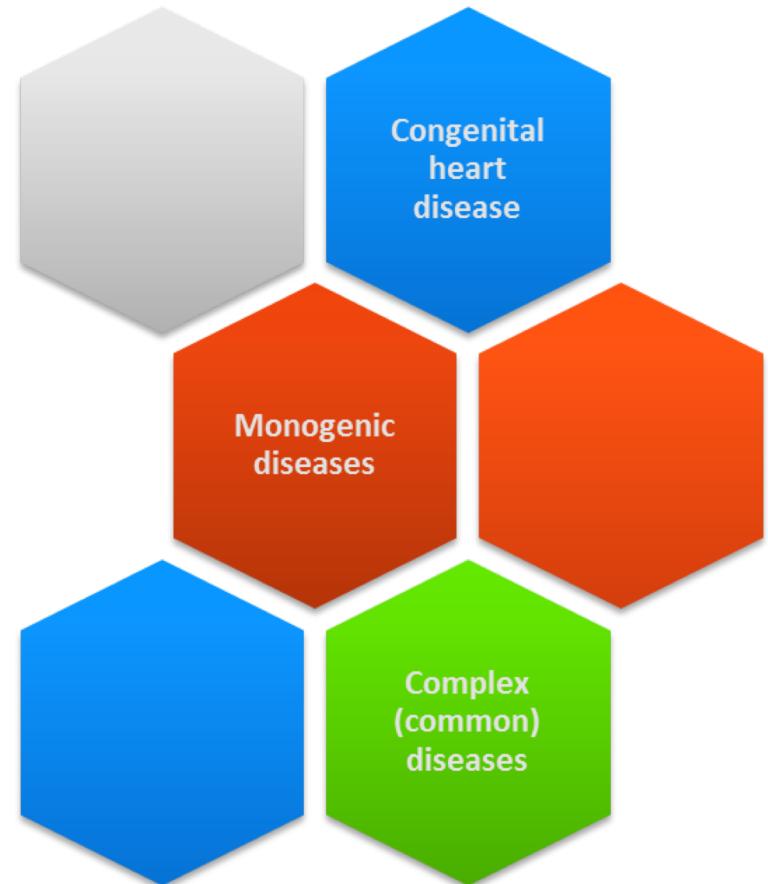
- Framingham Heart Study | www.framinghamheartstudy.org
 - A positive history of cardiovascular disease and associated risk factors tend to aggregate in families
 - Familial aggregation heritability of CVD estimated $\geq 90\%$ (before 46 years)
 - Family history is an independent risk factor (FHS)
 - 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?

- **Congenital heart disease**
 - Atrial, ventricular septal defects
 - Could be genetic, could be teratogen
- **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, e.g.:
 - Sex-determination (SRY)
 - Leber's hereditary optic neuropathy (LHON)



What type of disease are we looking at?

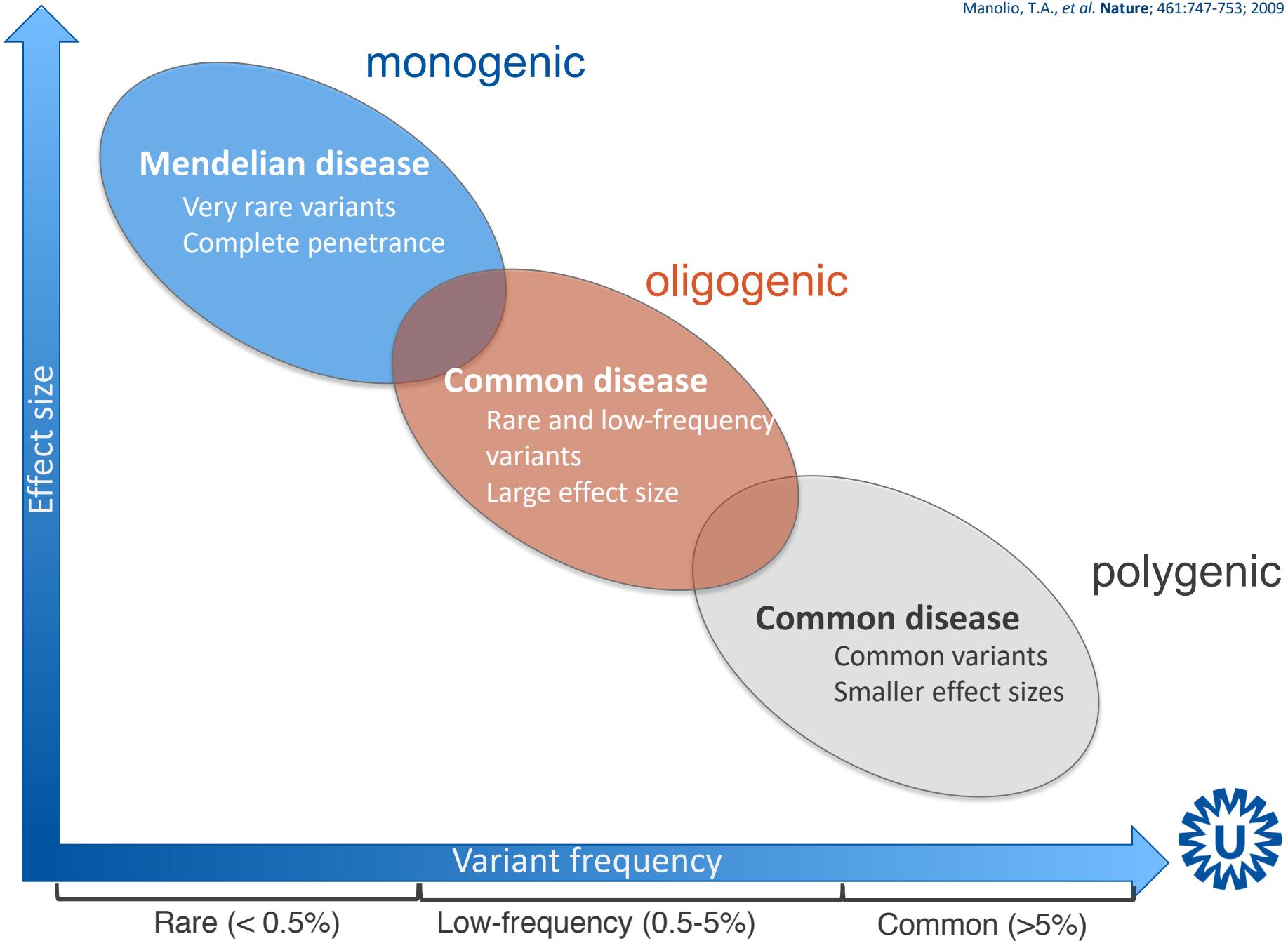
- **Monogenic diseases**
 - Mendelian pattern
 - Autosomal dominant
 - Autosomal recessive
 - X-linked
 - Y-linked/Mitochondrial

Dissected OMIM Morbid Map Scorecard (Updated May 24th, 2016) :

Class of phenotype	Phenotype	Gene*
Single gene disorders and traits	4,719	3,174
Susceptibility to complex disease or infection	700	499
"Nondiseases"	141	111
Somatic cell genetic disease	202	115

*Some genes may be counted more than once because mutations in a gene may cause more than one phenotype and the phenotypes may be of different classes (e.g., activating somatic BRAF mutation underlying cancer, [164757.0001](#) and germline BRAF mutation in Noonan syndrome, [164757.0022](#).)





Monogenic

Genotype → Disease

Polygenic

Genotypes → Disease

Environment



Monogenic disease

- Highly *penetrant* alleles are associated with monogenic, Mendelian diseases
- Penetrance: the proportion of individuals that carry a disease variant and that also express the disease

Genotype



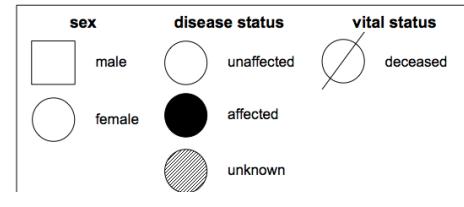
Disease



Environment

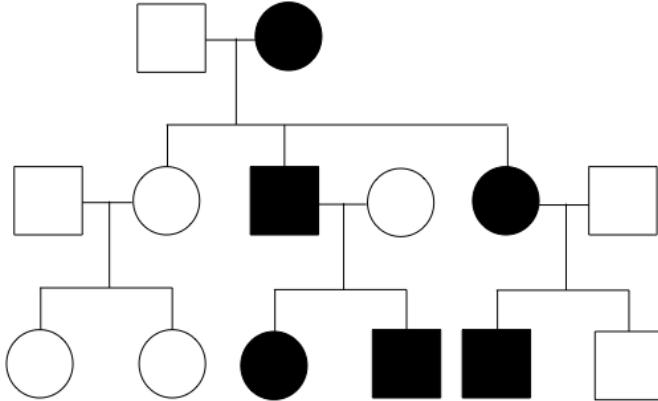


Key to pedigree charts



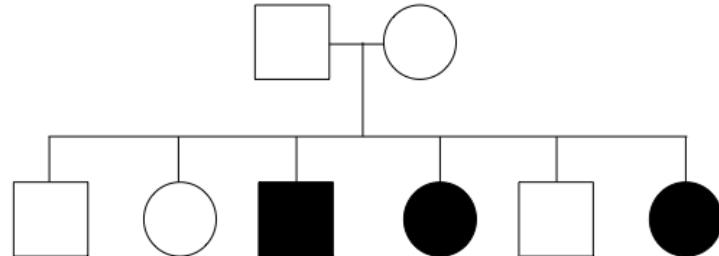
Types of inheritance

Autosomal dominant*

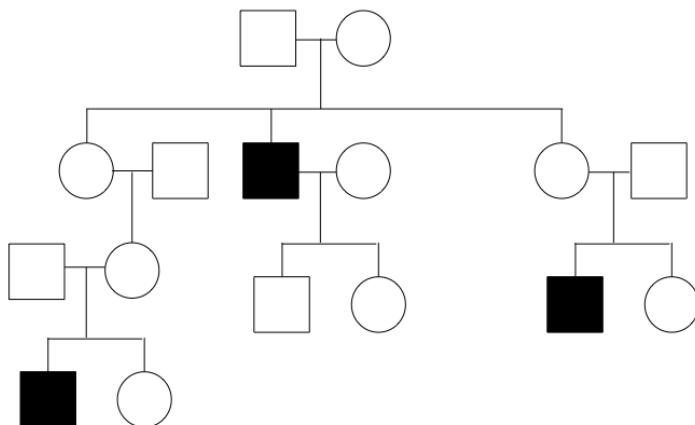


*note: if not for this one father-to-son transmission this arrow points to, it could also be X-Linked dominant

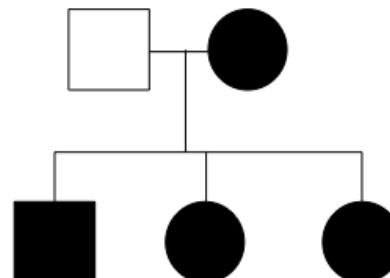
Autosomal recessive



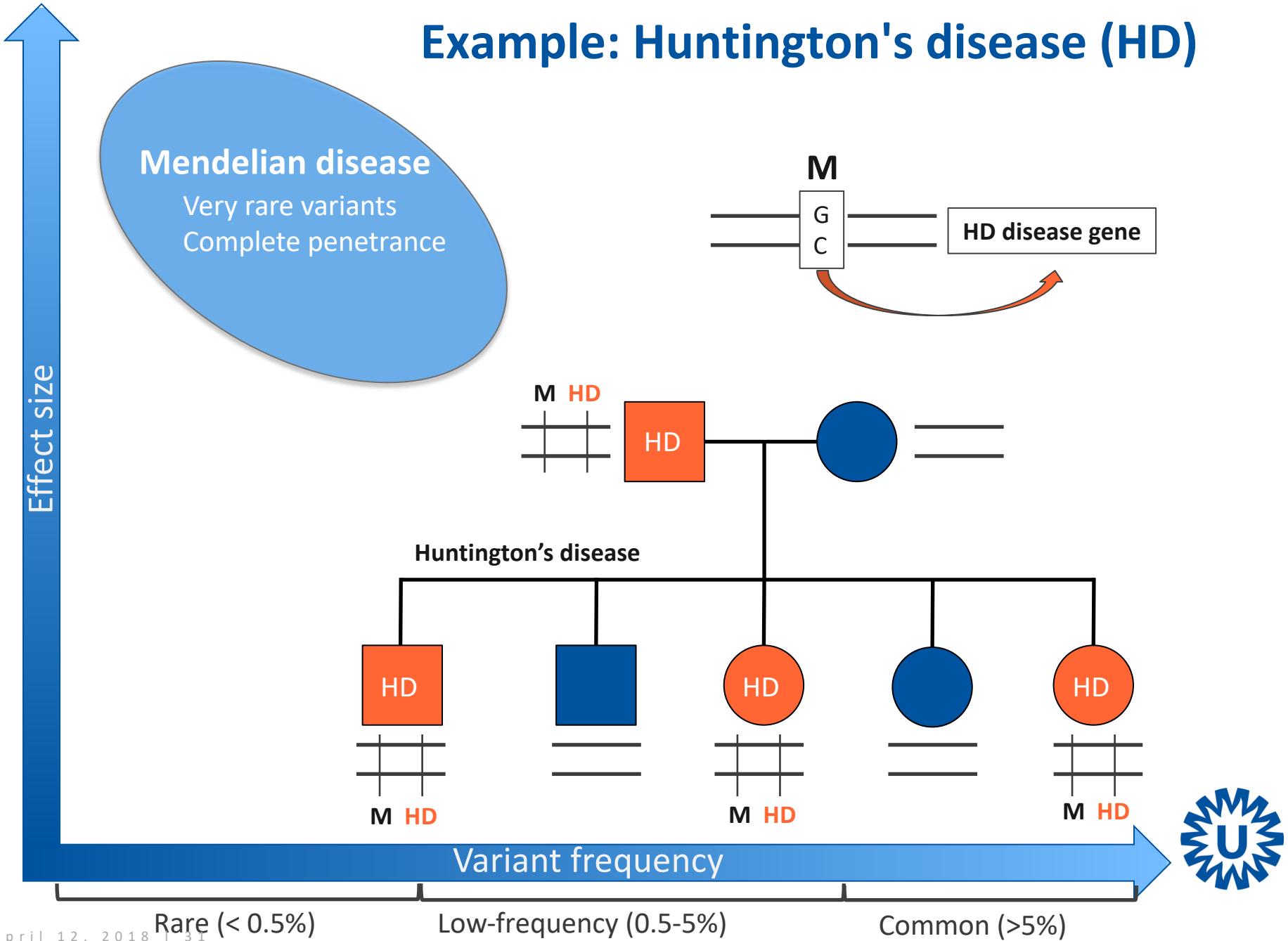
X-linked recessive



Mitochondrial



Example: Huntington's disease (HD)



A polymorphic DNA marker genetically linked to Huntington's disease

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

* Neurology Department and Genetics Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA

† Hereditary Disease Foundation, 9701 Wilshire Blvd, Beverly Hills, California 90212, USA

‡ Department of Medical Genetics, Indiana University Medical Center, Indianapolis, Indiana 46223, USA

§ Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York 14263, USA

|| Venezuela Collaborative Huntington's Disease Project*

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

1983



A polymorphic DNA marker genetically linked to Huntington's disease

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* Neurology Department and Genetics Unit, Massachusetts General Hospital, Boston, MA 02114

† Hereditary Disease Foundation, 9701 Wilshire Boulevard, Los Angeles, CA 90021

‡ Department of Medical Genetics, Indiana University Medical School, Indianapolis, IN 46285

§ Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, NY 14263
|| Venezuela Collaborative Huntington's Disease Study Group

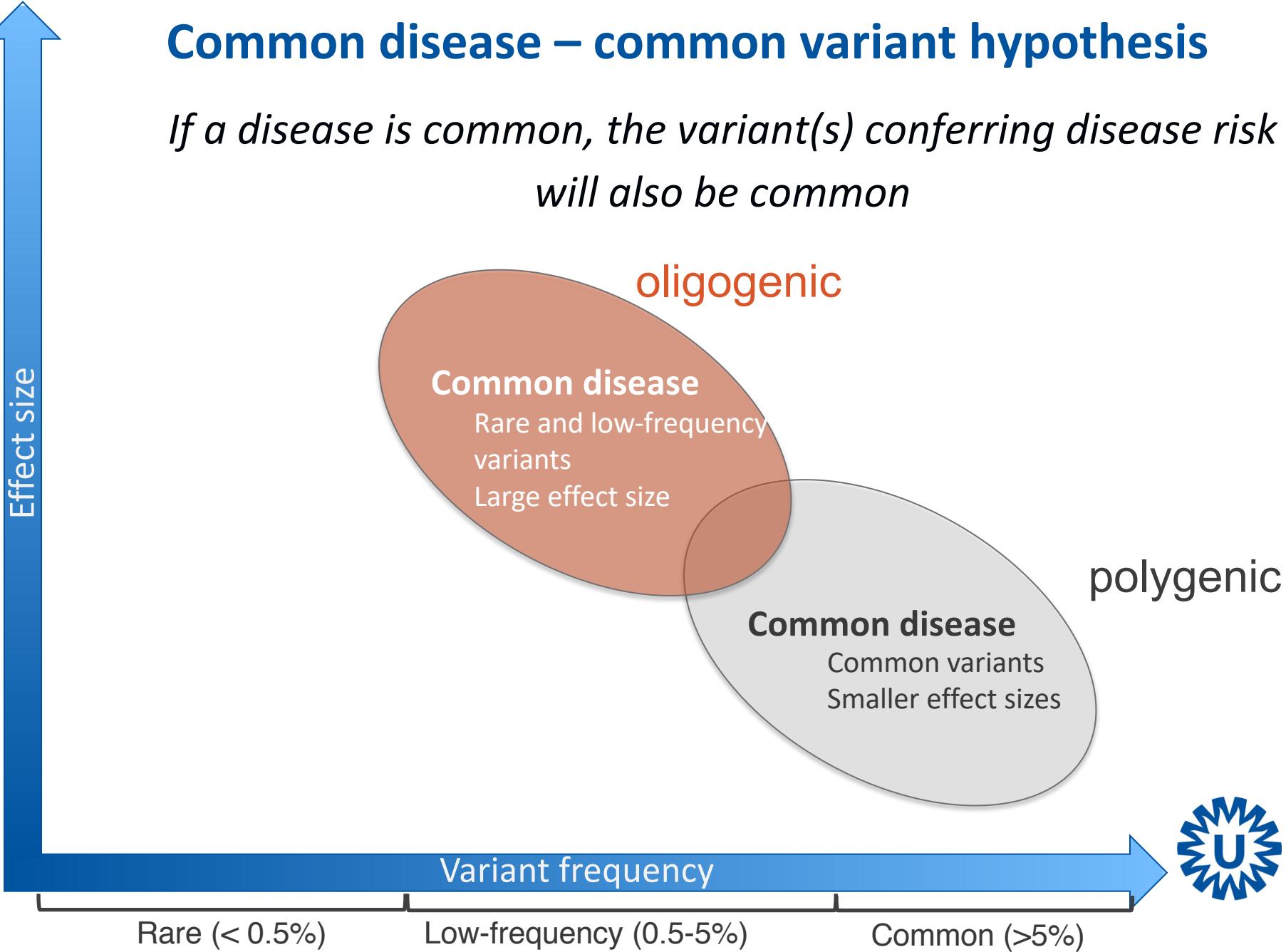
1983

1989



Common disease – common variant hypothesis

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



What type of disease are we looking at?

• Complex diseases

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

Each gene contributes a little to the disease

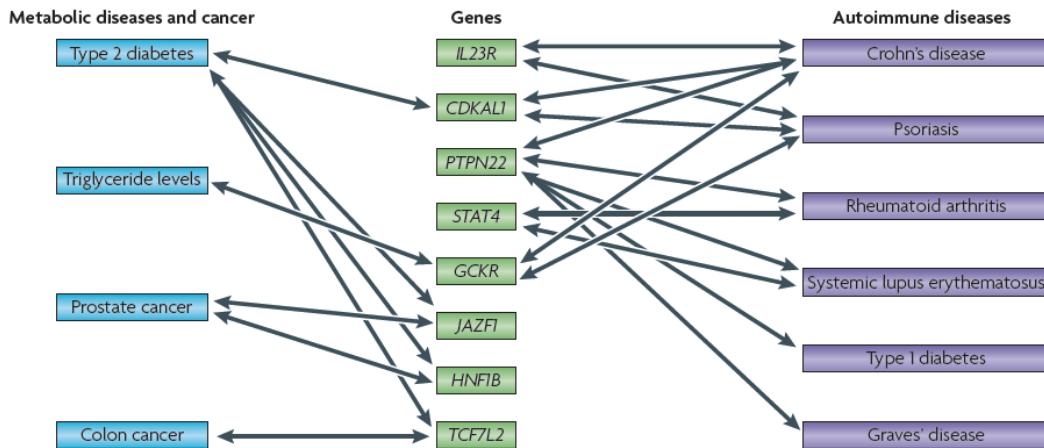


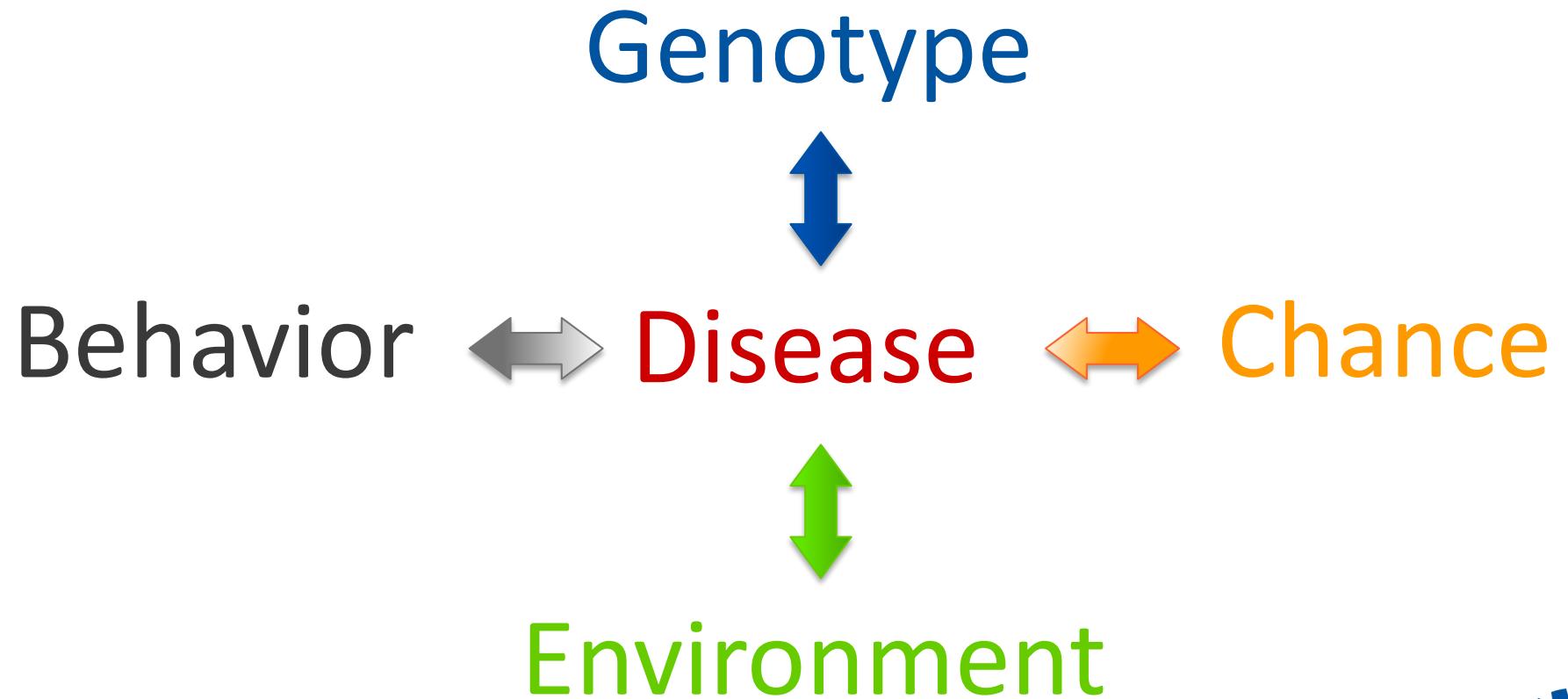
Table 3 Some Recent Genes/Loci Identified in Coronary Artery Disease

Gene/Locus	Functional Genomics	Independently Replicated	Reference(s)
CFH	Inflammation	Yes	49,50
LTA4H	Inflammation	No	12
FLAP	Inflammation	No	5
Lymphotxin 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.



Many factors influence complex traits and common disease



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 Valdimarsson³, Stefan E Matthiasson³, Halldor Johannsson³, Olof Guðmundsdottir¹, Mark E Gurney¹, Jesus Sainz¹, Margaret 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 Stefansson¹

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

Wellcome Trust Case-Control Consortium

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

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

- 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 Amaldrur 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 Guðmundur Þorgerðsson,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

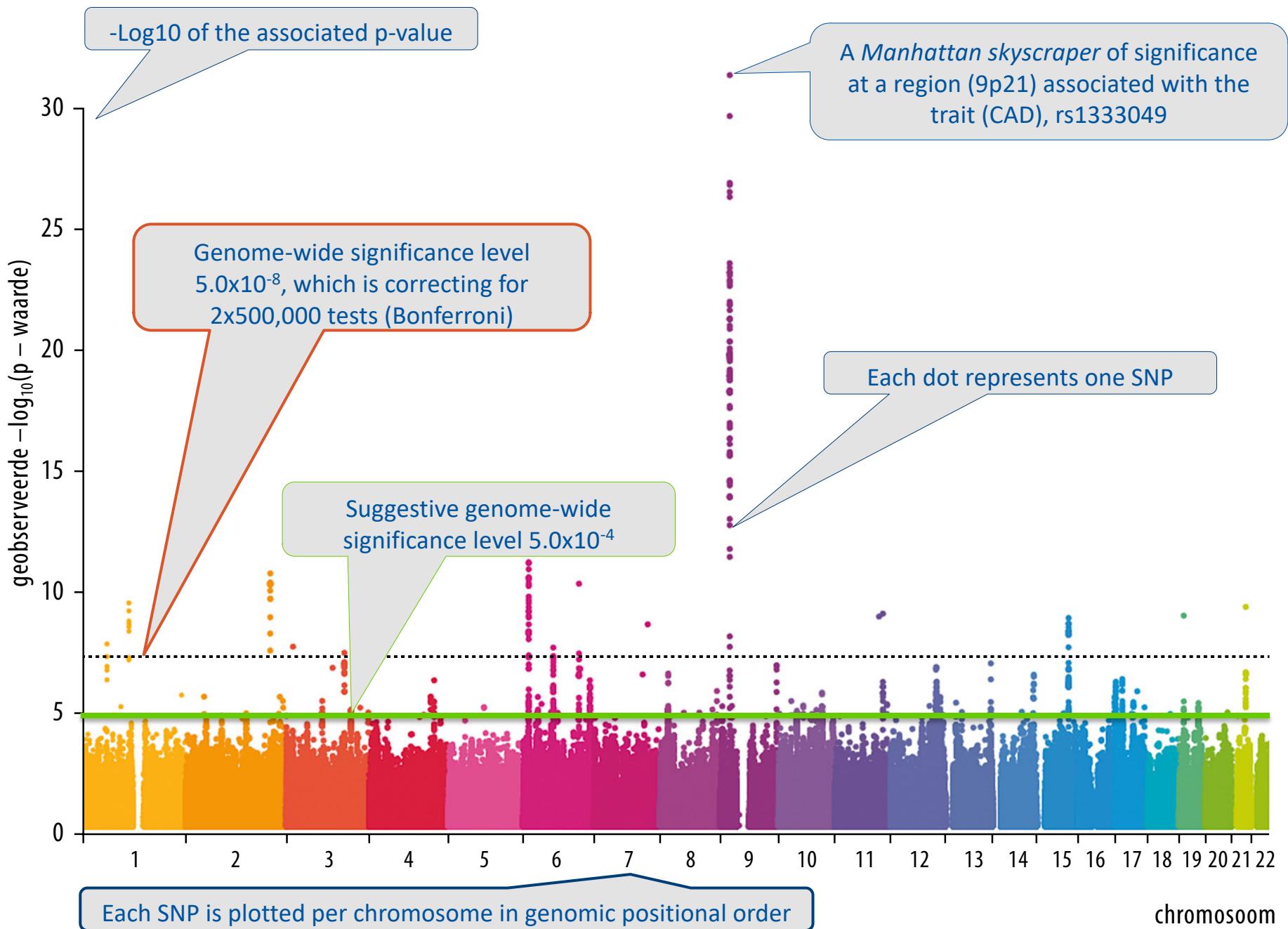


ARTICLES

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

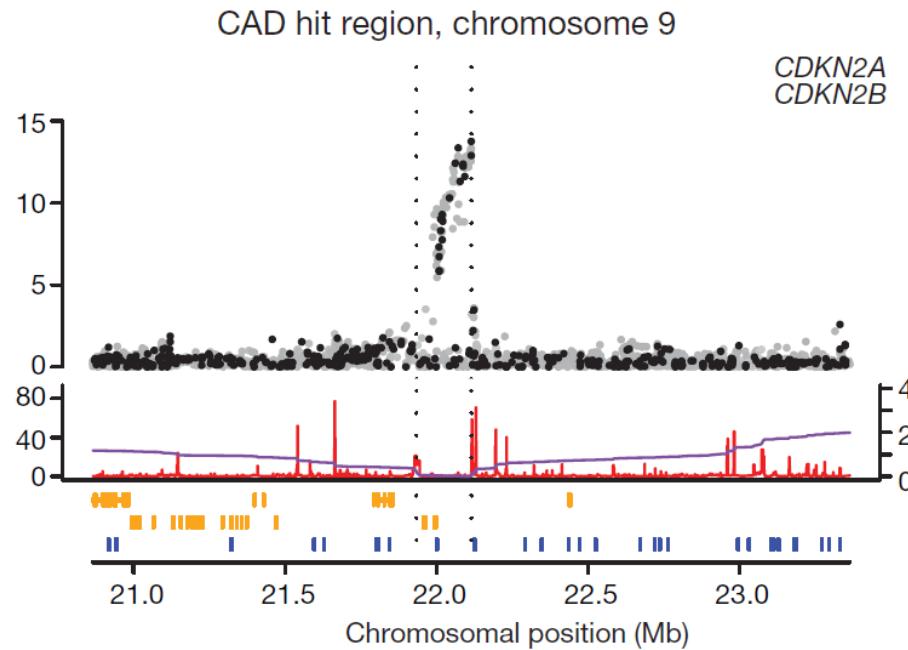
The Wellcome Trust Case Control Consortium*





9p21 and cardiovascular disease

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



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

A closer look at the results...

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

Collection	Chromosome	Region (Mb)	SNP	Trend P value	Genotypic P value	$\log_{10}(BF)$, 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

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

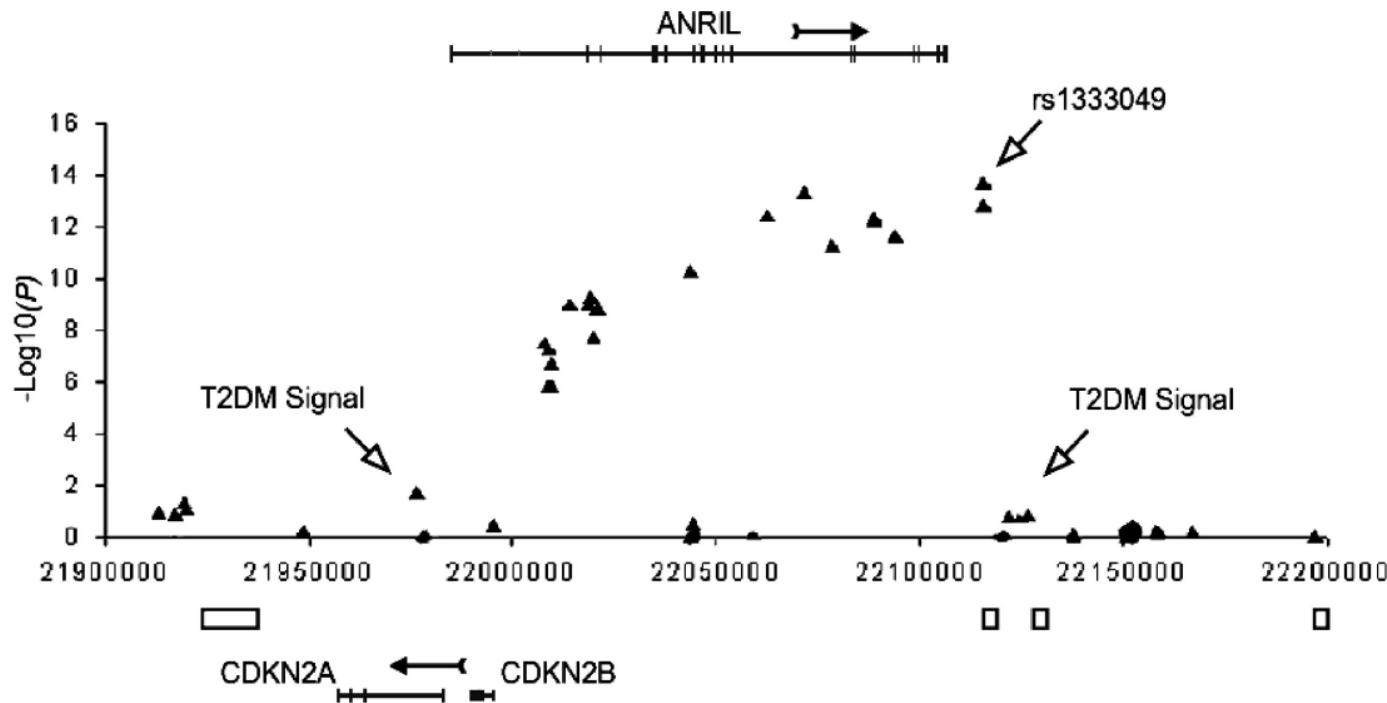
P-value of association test: AA vs. AB vs. BB

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



9p21 points to a RNA gene

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



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

CARDIoGRAMplusC4D Study

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



ARTICLES

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

The CARDIoGRAMplusC4D Consortium¹

Coronary artery disease (CAD) is the commonest cause of death. Here, we report an association analysis in 63,746 CAD cases and 130,681 controls identifying 15 loci reaching genome-wide significance, taking the number of susceptibility loci for CAD to 46, and a further 104 independent variants ($r^2 < 0.2$) strongly associated with CAD at a 5% false discovery rate (FDR). Together, these variants explain approximately 10.6% of CAD heritability. Of the 46 genome-wide significant lead SNPs, 12 show a significant association with a lipid trait, and 5 show a significant association with blood pressure, but none is significantly associated with diabetes. Network analysis with 233 candidate genes (loci at 10% FDR) generated 5 interaction networks comprising 85% of these putative genes involved in CAD. The four most significant pathways mapping these networks are linked to lipid metabolism and inflammation, underscoring the causal role of these activities in the genetic etiology of CAD. Our study provides insights into the genetic basis of CAD and identifies key biological pathways.

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NPG

Coronary artery disease and its main complication, myocardial infarction, is the leading cause of death worldwide. Although, epidemiological studies have identified many risk factors for CAD, including plasma lipid concentrations, blood pressure, smoking, diabetes and markers of inflammation, a causal role has been proven only for some (for example, low-density lipoprotein (LDL) cholesterol and blood pressure), primarily through randomized clinical trials of drug therapy directed at the risk factor¹. Twin and family studies have documented that a significant proportion (40–50%) of susceptibility to CAD is heritable (for a review, see ref. 2). Because genotypes are not confounded by environmental exposures, genetic analysis has the potential to define which risk factors are indeed causal and to identify pathways and therapeutic targets^{3,4}. To date, genome-wide association studies (GWAS) have collectively reported a total of 31 loci, associated with CAD risk at genome-wide significance ($P < 5 \times 10^{-8}$)^{5–13}. However, variants at these loci explain less than 10% of the heritability of CAD. One likely reason for this is that, given the polygenic nature of complex traits and the relatively small observed effect sizes of the loci identified, many genuinely associated variants do not reach the stringent P -value threshold for genome-wide significance. Indeed, there is increasing evidence that the genetic architecture of common traits involves a large number of causative alleles with very small effects¹⁴. Addressing this will require the discovery of additional loci while leveraging large-scale genomic data to identify the molecular pathways underlying the pathogenesis of CAD. Such discovery is facilitated by building molecular networks, on the basis of DNA, RNA and protein interactions, which have nodes of known biological function that also show evidence of association with risk variants for CAD and related metabolic traits.

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

Meta-analysis (CARDIoGRAM) Consortium⁵, which involved 22,233 cases and 64,762 controls, in addition to loci reported at genome-wide significance, a linkage disequilibrium (LD)-pruned set of 6,222 variants achieved a nominal association P value of less than 0.01. Here, we test these 6,222 SNPs in a meta-analysis of over 190,000 individuals, with the primary aim of identifying additional susceptibility loci for CAD. To this end, we used the Metabochip array¹⁵, which is a custom iSELECT chip (Illumina) containing 196,725 SNPs, designed to (i) follow-up putative associations in several cardiometabolic traits, including CAD, and (ii) fine map confirmed loci for these traits. All SNPs on the array with data in the CARDIoGRAM study were considered for analysis (79,138 SNPs, of which 6,222 were the replication SNPs and 20,876 were fine-mapping SNPs in the 22 CAD susceptibility loci identified at the time at which the array was designed; the remaining SNPs were submitted by the other consortia contributing to the Metabochip array¹⁵). In addition, we assess whether the genome-wide significant CAD risk alleles act through traditional risk factors by considering the available large GWAS for these traits^{16–20}. Finally, we identify a broader set of SNPs passing a conservative FDR threshold for association with CAD and use this set to undertake network analysis to find key biological pathways underlying the pathogenesis of CAD.

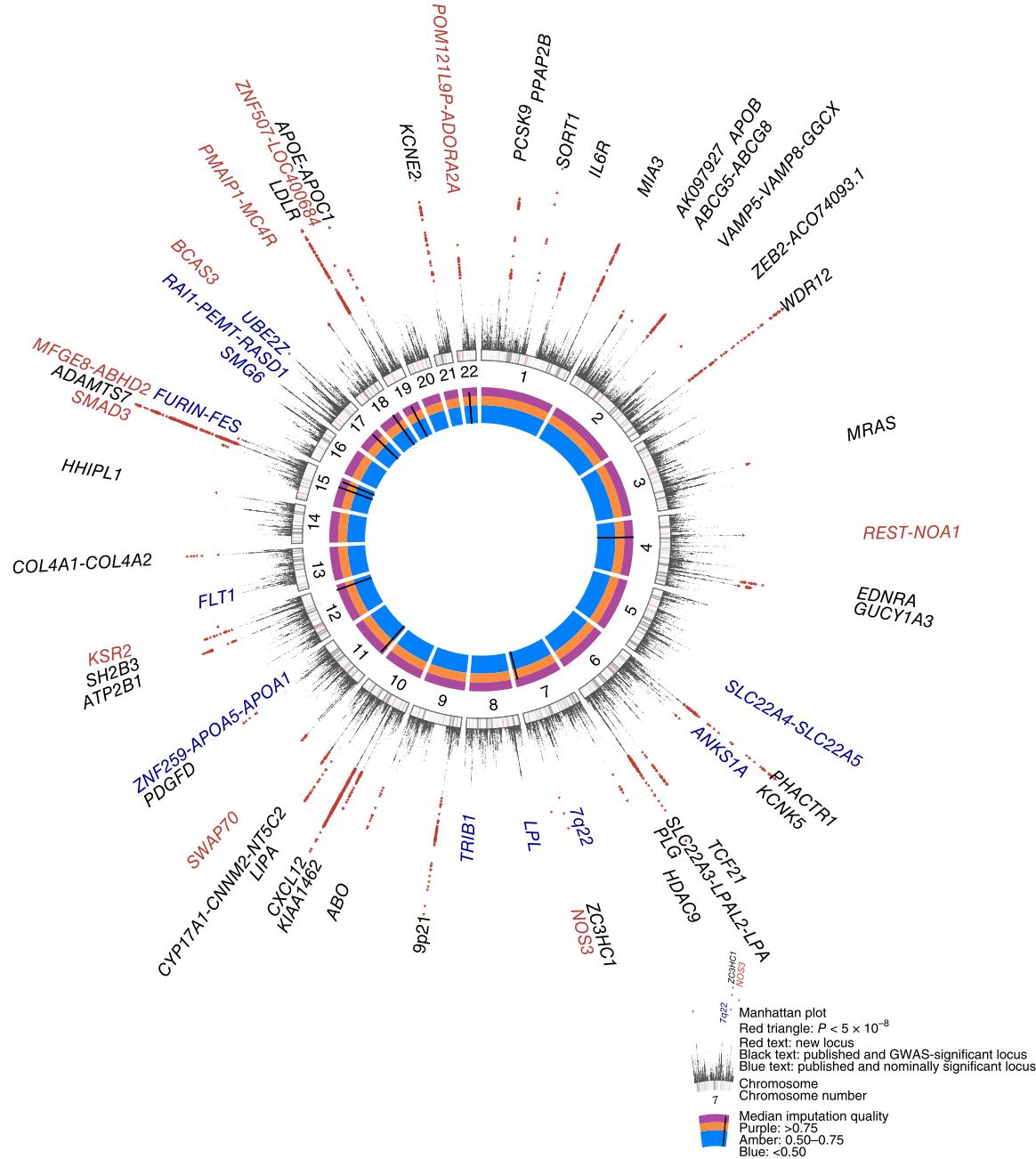
RESULTS Study design

We expanded the CARDIoGRAM discovery data set (22,233 cases and 64,762 controls⁵; stage 1) with 34 additional CAD sample collections (stage 2) of European or south Asian descent comprising 41,513 cases and 65,919 controls (study descriptions and sample characteristics are given in **Supplementary Tables 1a** and **2a**, respectively) and undertook a 2-stage meta-analysis to test SNPs on the Metabochip array

¹A full list of authors and affiliations appears at the end of the paper.

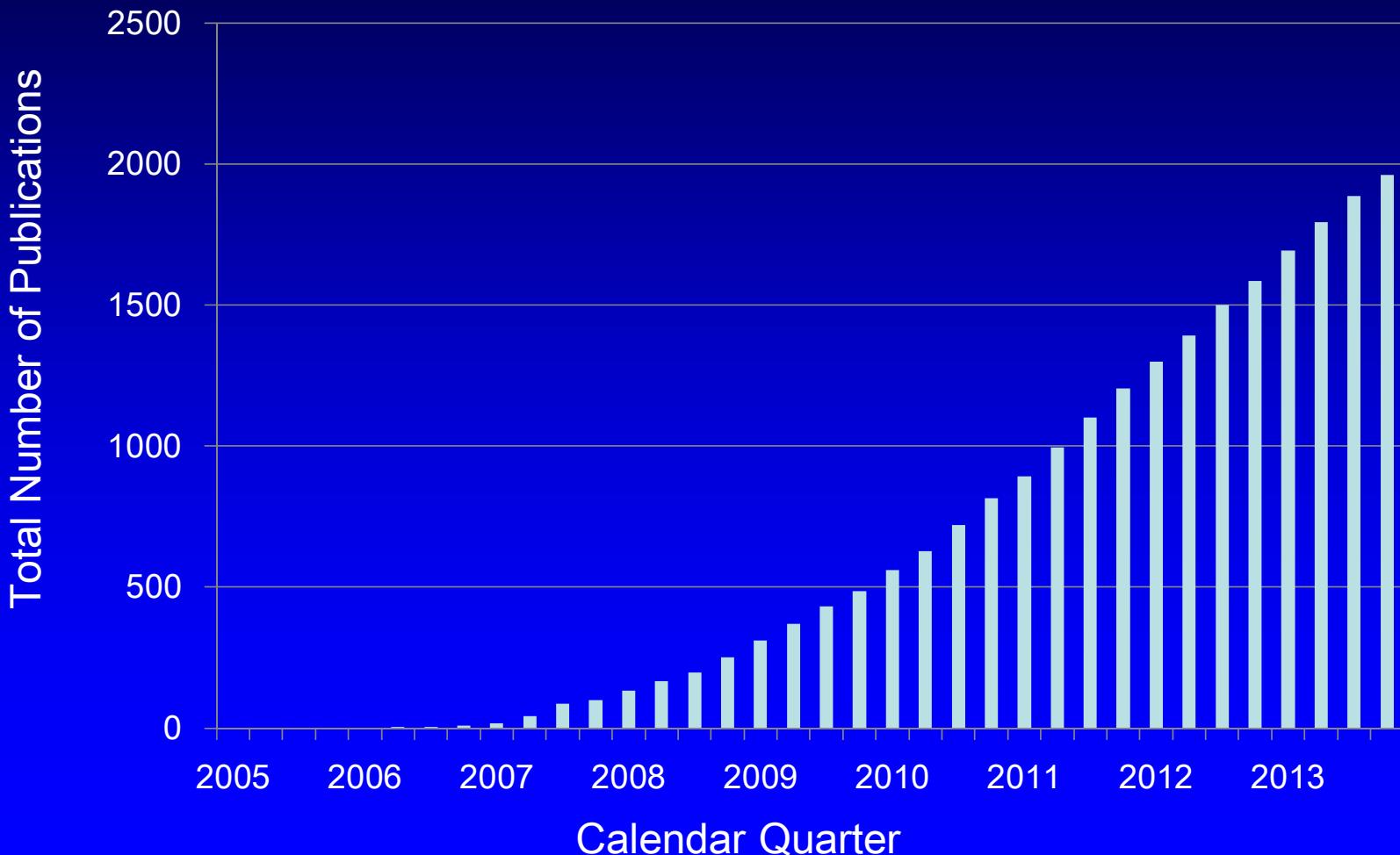
Received 24 April; accepted 2 November; published online 2 December 2012; doi:10.1038/ng.2480





Published GWA Reports, 2005 – 2013

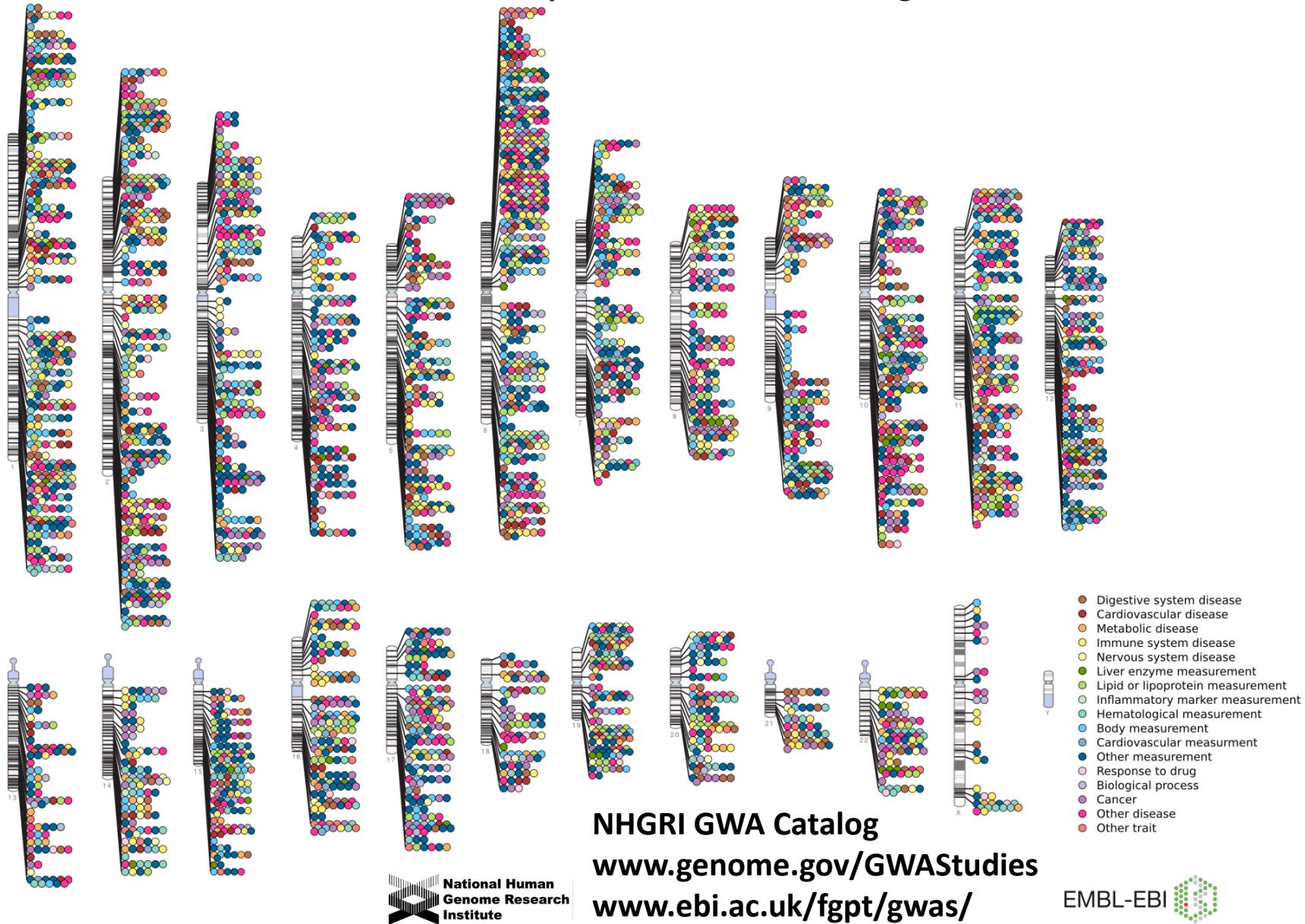
1960



Through 9/30/10 postings

Published Genome-Wide Associations through 12/2013

Published GWA at $p \leq 5 \times 10^{-8}$ for 17 trait categories



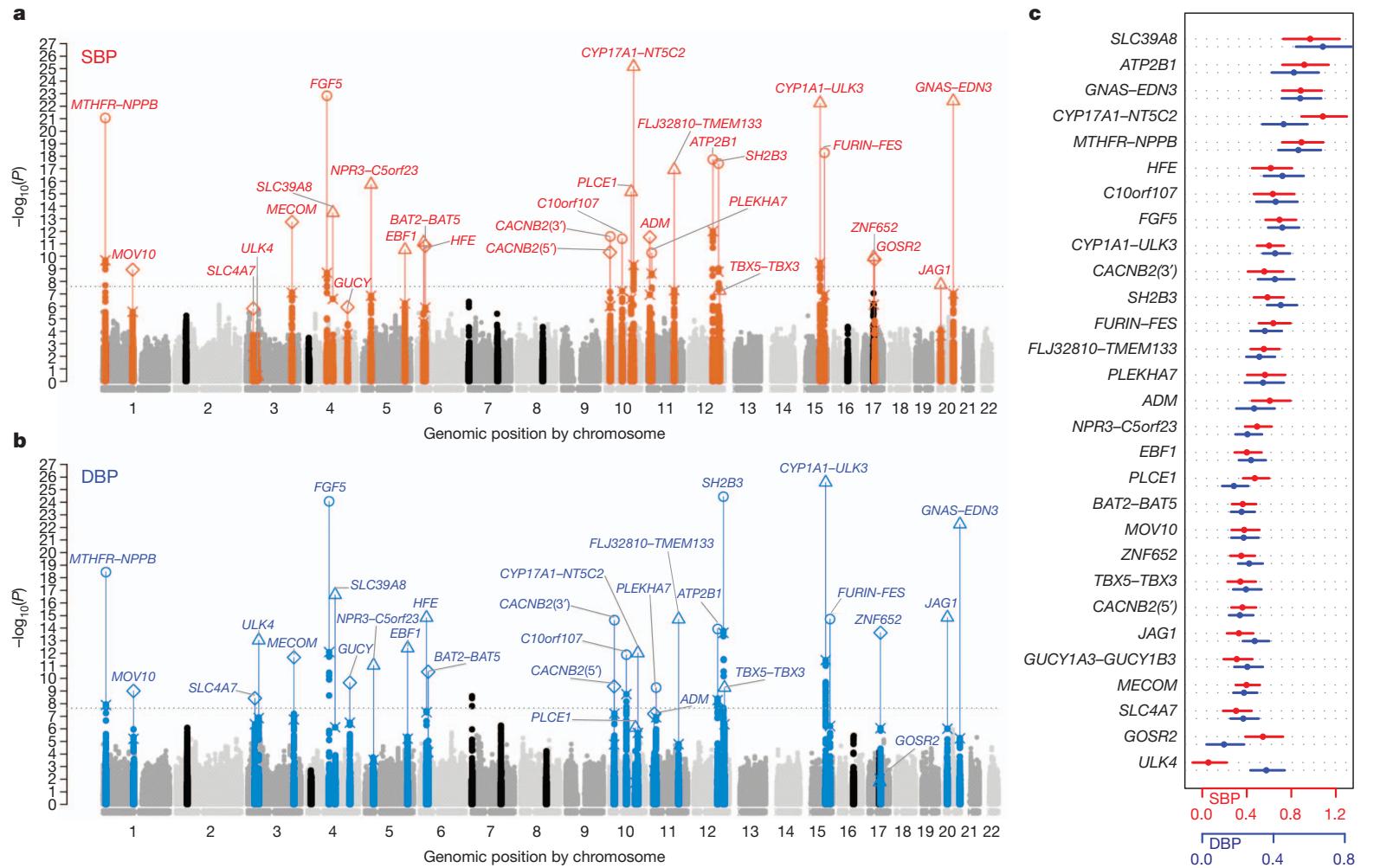


Figure 1 | Genome-wide $-\log_{10} P$ -value plots and effects for significant loci. **a, b**, Genome-wide $-\log_{10} P$ -value plots are shown for SBP (a) and DBP (b). SNPs within loci reaching genome-wide significance are labelled in red for SBP and blue for DBP (± 2.5 Mb of lowest P value) and lowest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

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



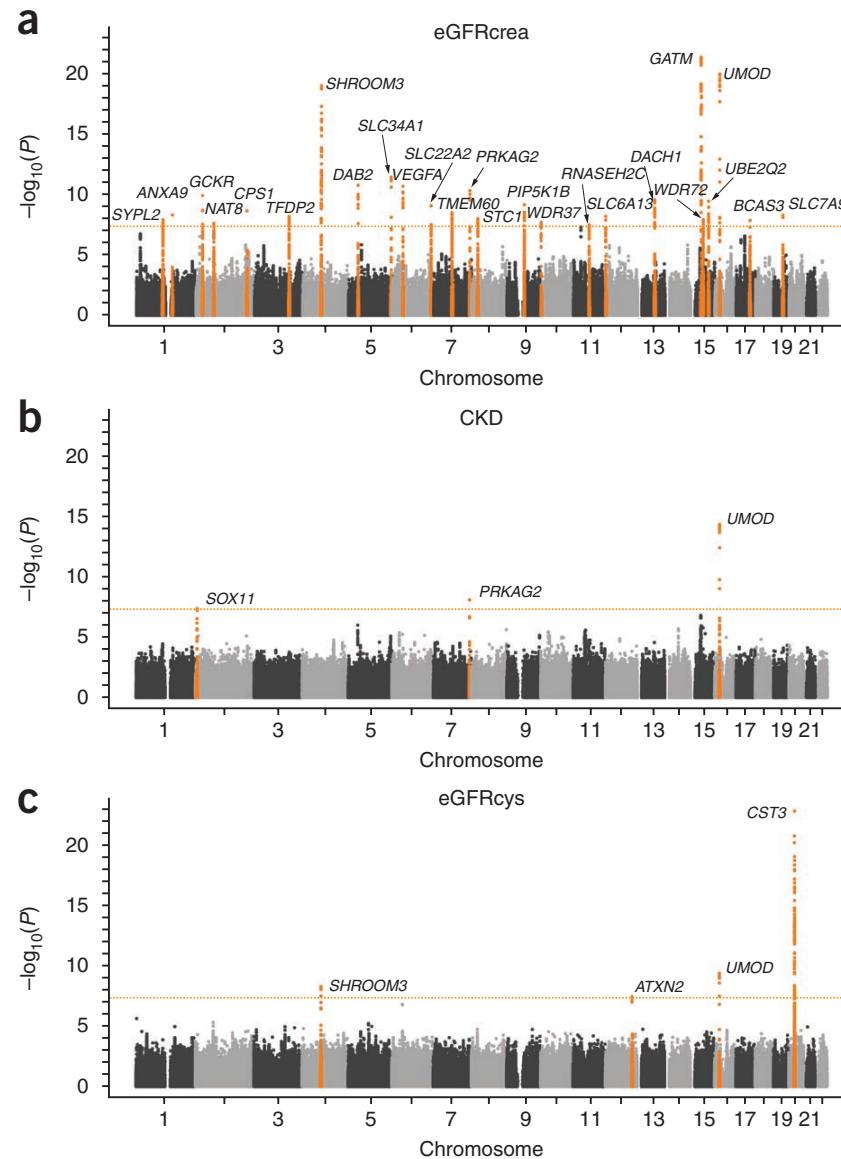


Figure 1 Genome-wide $-\log_{10} P$ value plot from stage 1. (a–c) Plots show discovery analysis of eGFRcrea (a), CKD (b) and eGFRcys (c). The dotted line indicates the genome-wide significance threshold at $P = 5 \times 10^{-8}$.



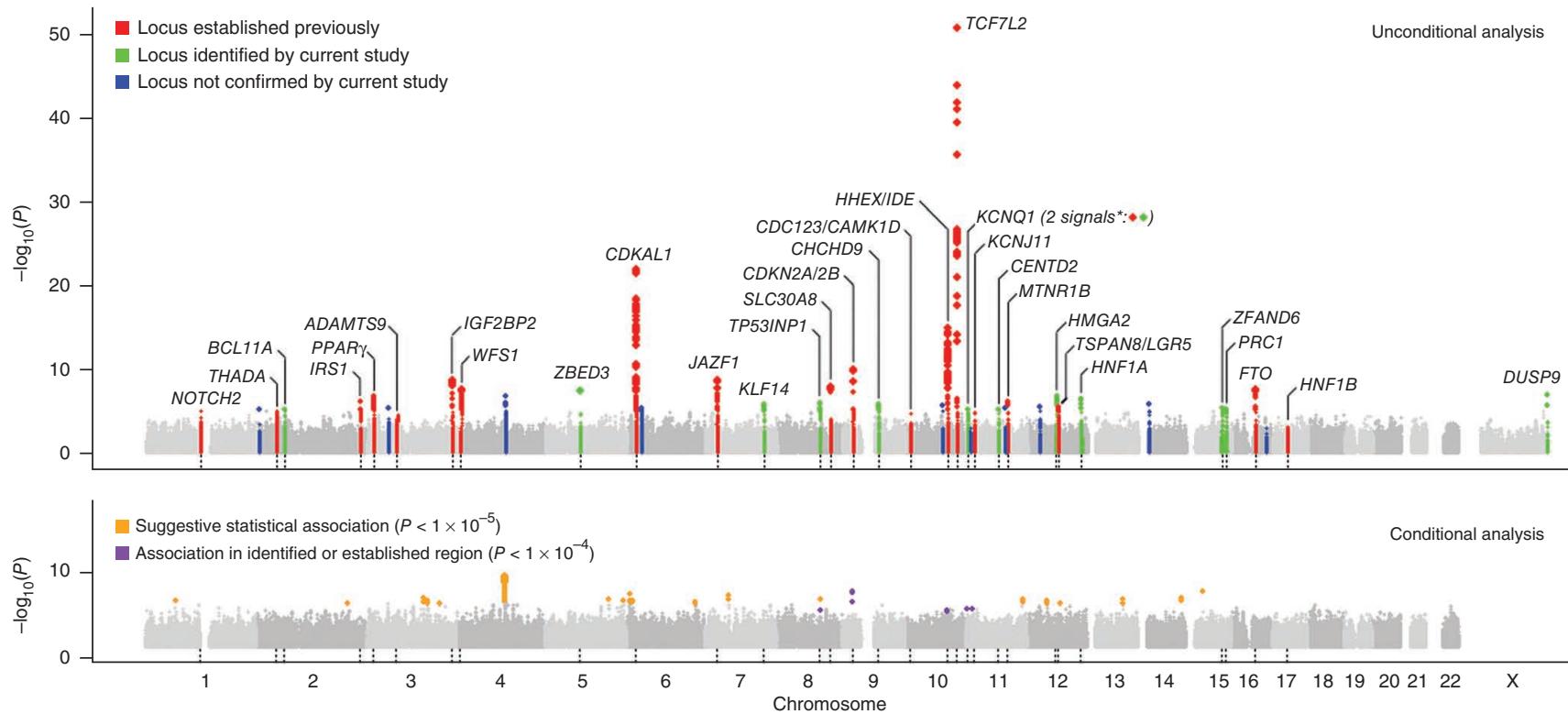
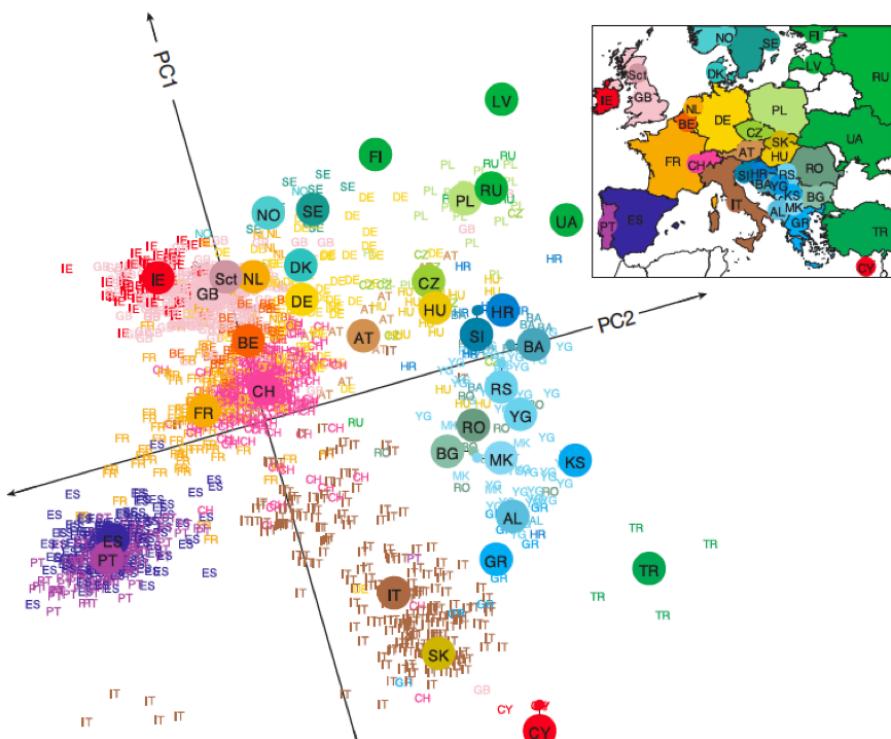
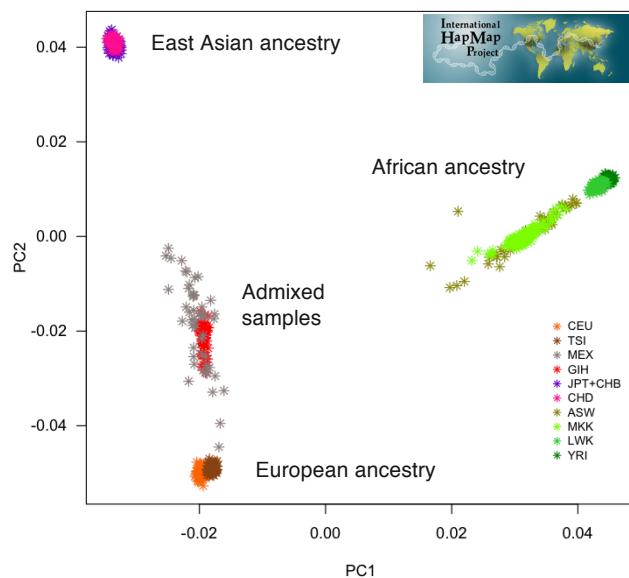


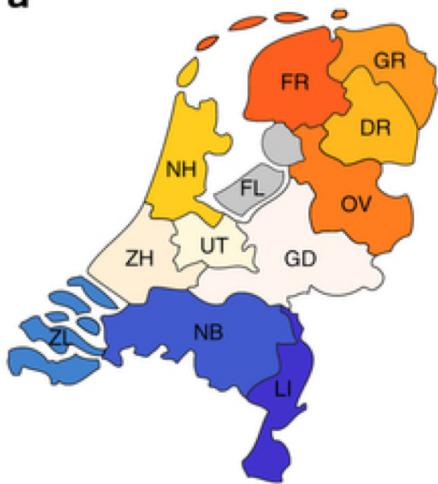
Figure 1 Genome-wide Manhattan plots for the DIAGRAM+ stage 1 meta-analysis. Top panel summarizes the results of the unconditional meta-analysis. Previously established loci are denoted in red and loci identified by the current study are denoted in green. The ten signals in blue are those taken forward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



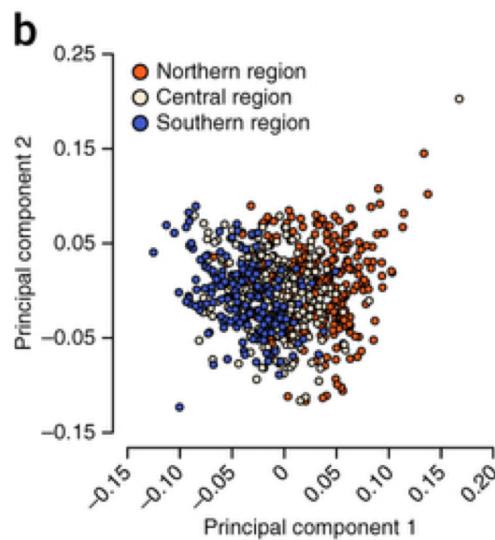
Population stratification



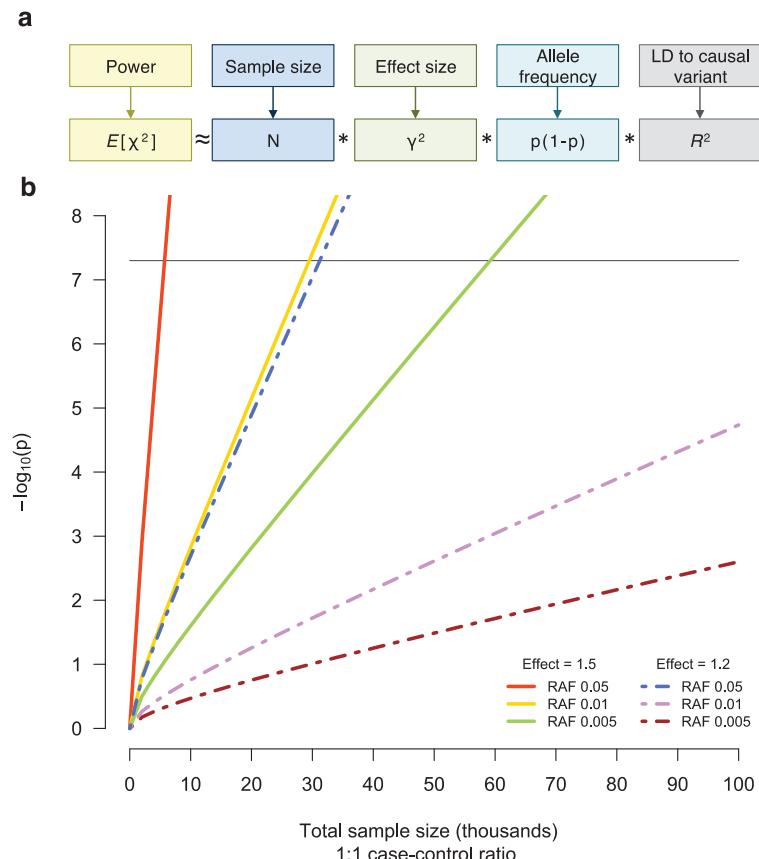
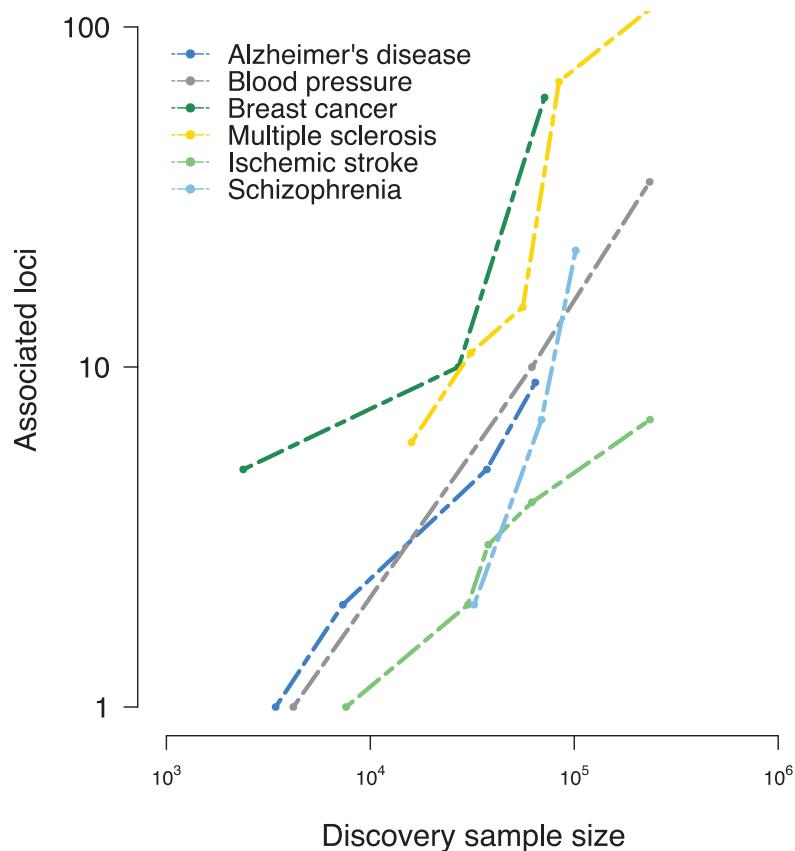
a



b



Power, Effect size, Sample size...



Cardiovascular Genetic Research

Experimental Cardiology Laboratory

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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

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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.

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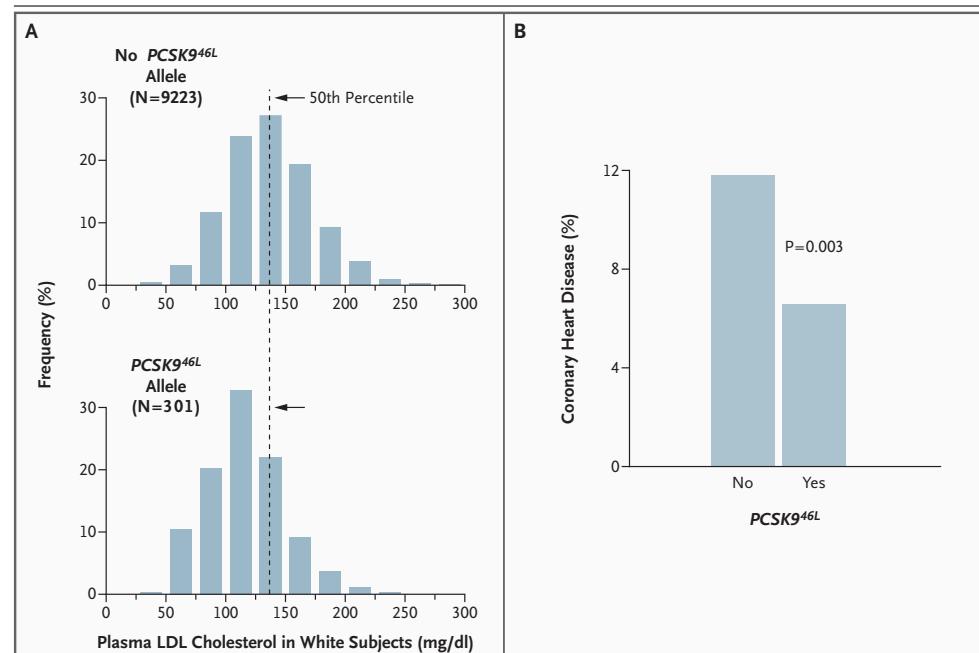
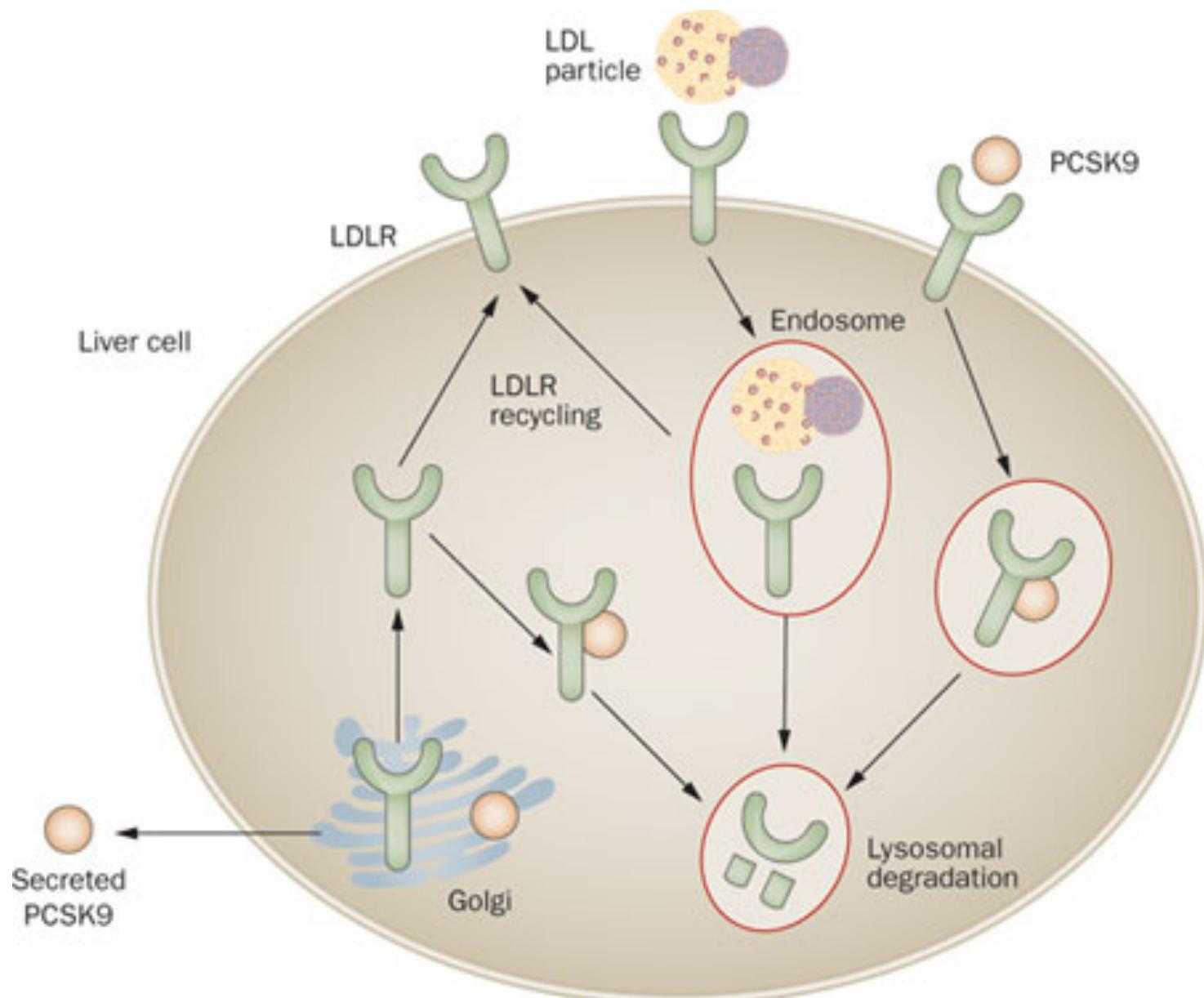


Figure 2. Distribution of Plasma LDL Cholesterol Levels (Panel A) and Incidence of Coronary Events (Panel B) among White Subjects, According to the Presence or Absence of a PCSK9^{46L} Allele.

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

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William B. Smith, M.D., Eleanor Lisbon, M.D., M.P.H., Maria Gutierrez, M.D.,
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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.

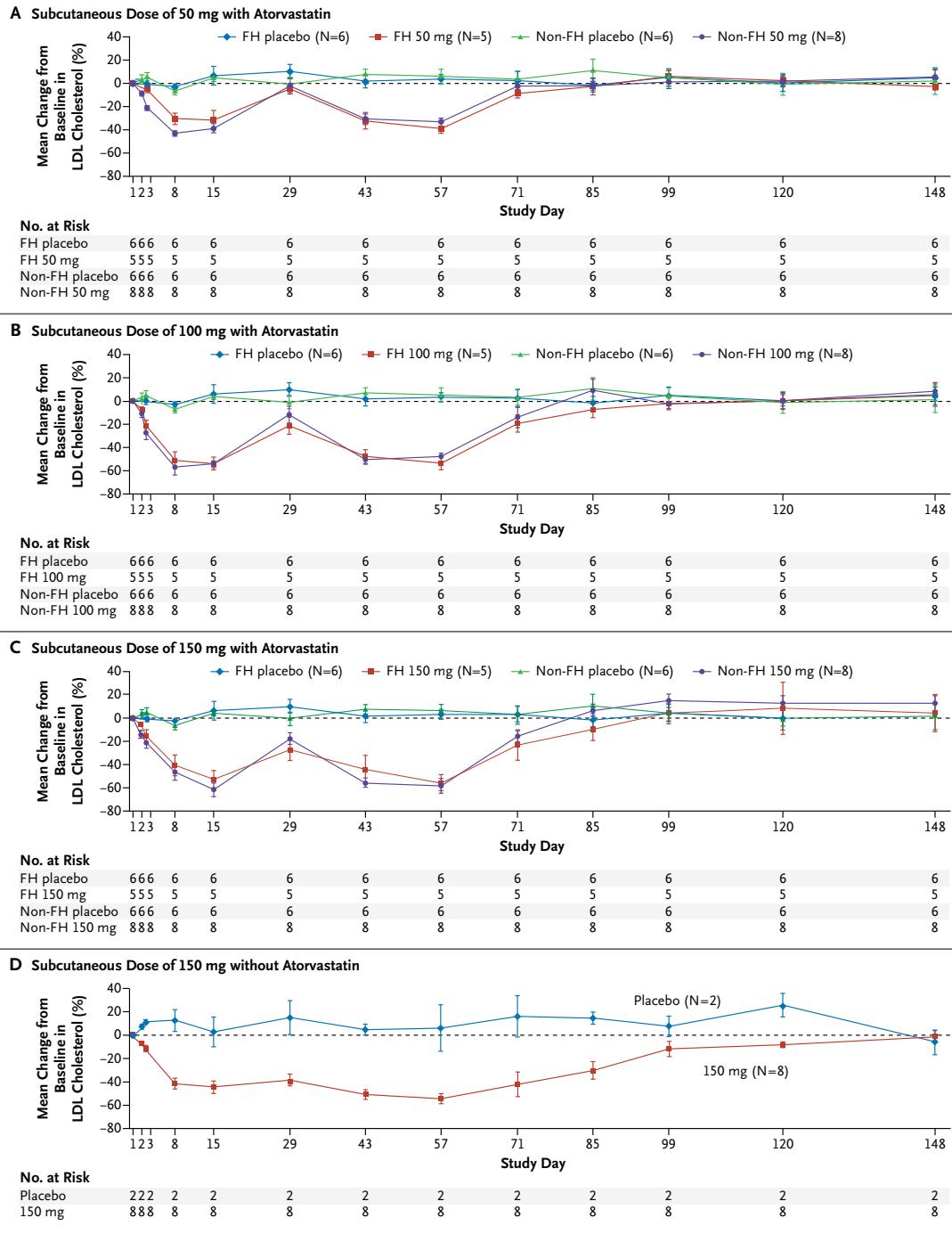
RESULTS

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.)





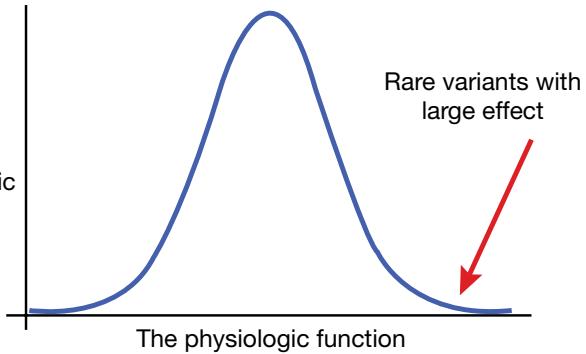
Human genetics as a foundation for innovative drug development

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.