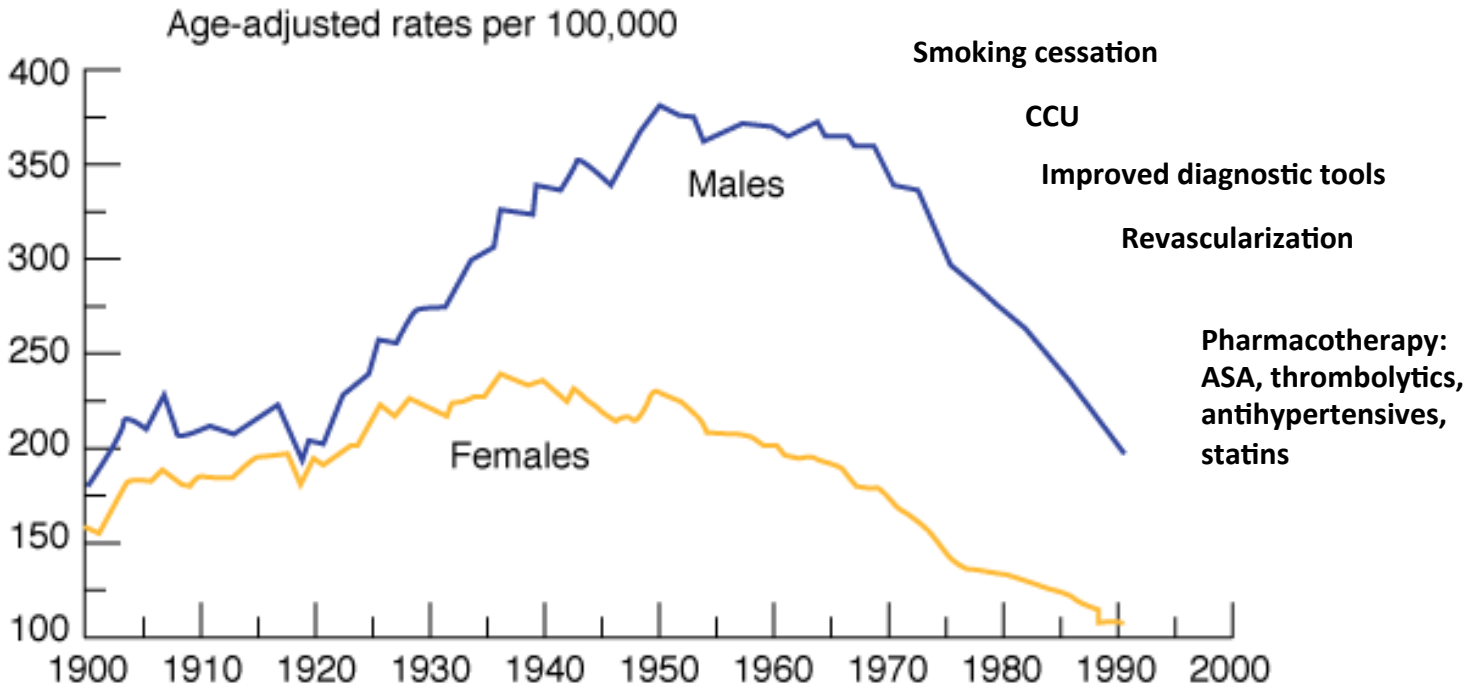
Genetics of Coronary Artery Disease (CAD) Recent progress and challenges ahead

Themistocles (Tim) Assimes, MD PhD
Assistant Professor of Medicine
Stanford University School of Medicine

Outline

- Epidemiology of Coronary artery disease
- Pathophysiology of coronary atherosclerosis
  - "CAD", hardening of arteries
- Heritability of CHD and risk factors
- GWAS discoveries
- Current risk prediction for CHD
  - Clinical considerations
- Old and new metrics for assessing improvement in risk prediction algorithms
  - Bringing in genetics
- Future Directions

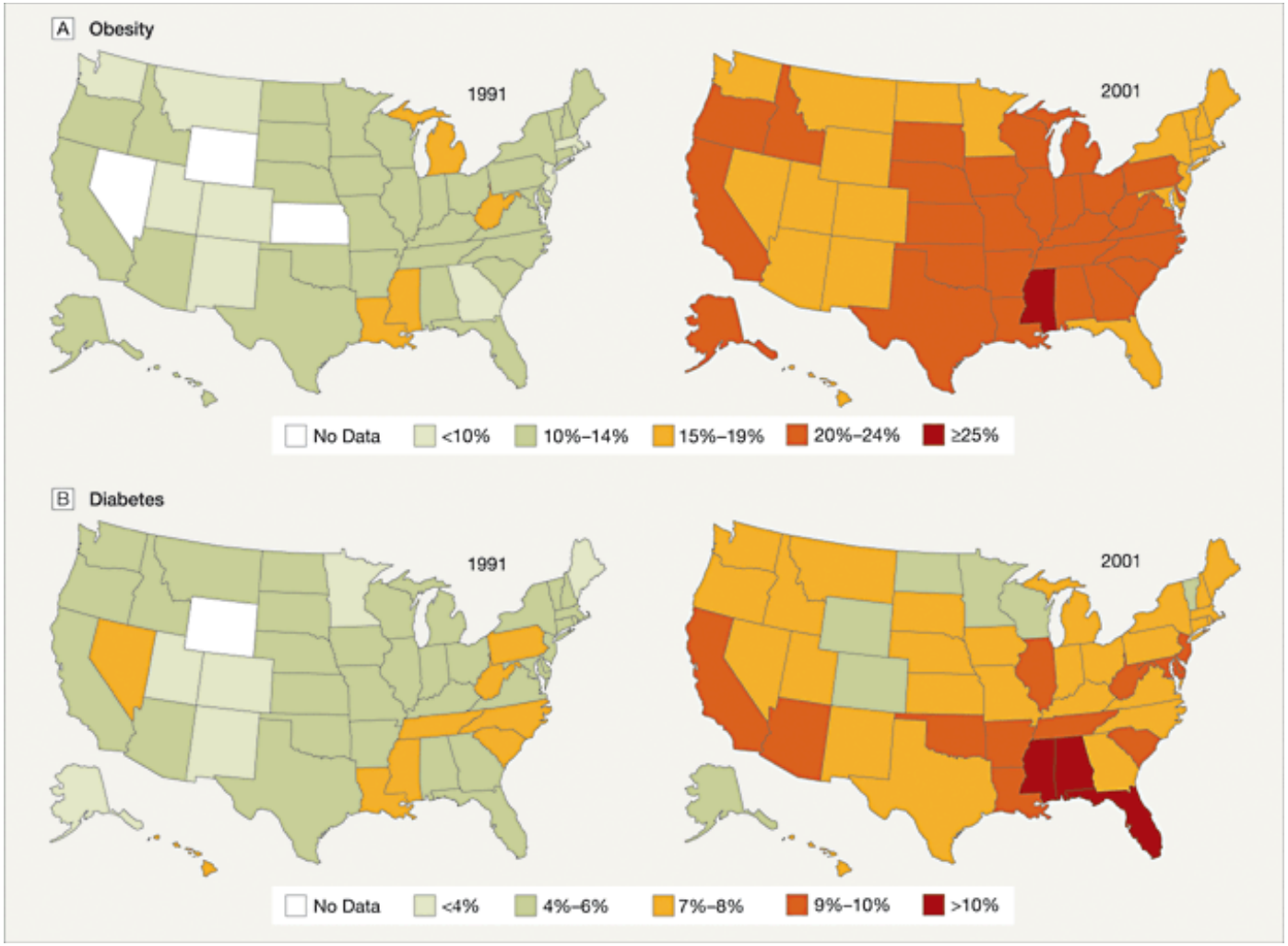
Trends in Death Rates for Heart Disease



Prevalence going up: baby boomers and…

Lippincott, Williams & Wilkins. Feinleib M. Trends in heart disease in the United States. Am J Med Sci 1995;310: s8-s14

Prevalence of Obesity and Diagnosed Diabetes Among US Adults, 1991 and 2001



Mokdad, A. H. et al. JAMA 2003;289:76-79.

JAMA

TABLE 1-2 -- FOUR TYPICAL STAGES OF THE EPIDEMIOLOGICAL TRANSITION

STAGE	DESCRIPTION	TYPICAL PROPORTION OF DEATHS DUE TO CVD (%)	PREDOMINANT TYPES OF CVD
Pestilence and famine	Predominance of malnutrition and infectious diseases as causes of death; high rates of infant and child mortality; low mean life expectancy	<10	Rheumatic heart disease cardiomyopathies due to infection and malnutrition
Receding pandemics	Improvements in nutrition and public health lead to decrease in rates of deaths due to malnutrition and infection; precipitous decline in infant and child mortality rates	10–35	Rheumatic valvular disease, hypertension, CHD, stroke
Degenerative and man-made diseases	Increased fat and caloric intake and decreased physical activity lead to emergence of hypertension and atherosclerosis; with increased life expectancy, mortality from chronic, noncommunicable diseases exceeds mortality from malnutrition and infectious diseases	35–65	CHD, stroke
Delayed degenerative diseases	Cardiovascular diseases and cancer are the major causes of morbidity and mortality; better treatment and prevention efforts help avoid deaths among those with disease and delay primary events. Age-adjusted CVD mortality declines; CVD affecting older and older individuals	50	CHD, stroke, congestive heart failure

CHD=coronary heart disease; CVD=cardiovascular disease.

Adapted from Omran AR: The epidemiologic transition: A theory of the epidemiology of population change. Milbank Mem Fund Q 49:509–538, 1971; and Olshansky SJ, Ault AB: The fourth stage of the epidemiologic transition: The age of delayed degenerative diseases. Milbank Q 64:355–391, 1986.

TABLE 1-1 -- BURDEN OF DISEASE (1990 ESTIMATES) FOR THE THREE ECONOMIC REGIONS OF THE WORLD

REGION	POPULATION (MILLIONS) (% TOTAL WORLD POPULATION)	% OF DEATHS IN THE REGION DUE TO			
		Cardiovascular Disease	Other Noncommunicable Diseases *	Communicable Diseases	Injuries
Developed					
EstME †	798 (15.2)	44.60%	42.80%	6.40%	10.70%
EmgME ‡	346 (6.6)	54.60%	29.50%	5.60%	10.30%
Developing					
DevE §	4124 (78.3)	23.00%	24.30%	46.90%	6.20%
Totals	5267	28.40%	27.40%	34.20%	10.10%

Adapted from Murray CJL, Lopez AD: The Global Burden of Disease. Cambridge, MA, Harvard School of Public Health, 1996.

*Includes cancer, diabetes, neuropsychiatric conditions, congenital anomalies, and respiratory, digestive, genitourinary, and musculoskeletal diseases.

† EstME: Established market economies—United States, Canada, Western Europe, Japan, Australia, and New Zealand.

‡ EmgME: Emerging market economies—former socialist states of Russian Federation.

§ DevE: Developing market economies—China, India, other Asia and islands, sub-Saharan Africa, Middle Eastern Crescent, Latin America and the Caribbean.

Bottom line: coronary heart disease is number 1 killer in the world and will continue to be number 1 killer as many countries transition

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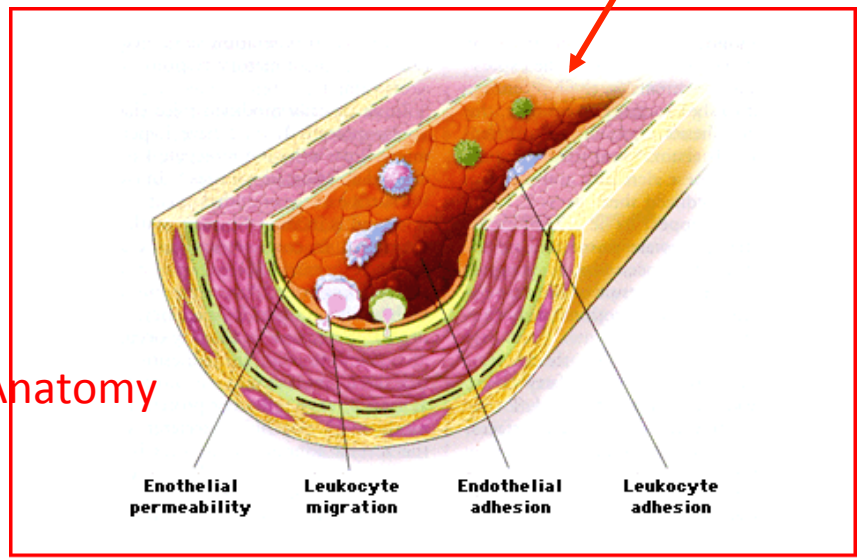
Crash Course for Atherosclerosis

In blood: cholesterol packets, WBC, clotting proteins

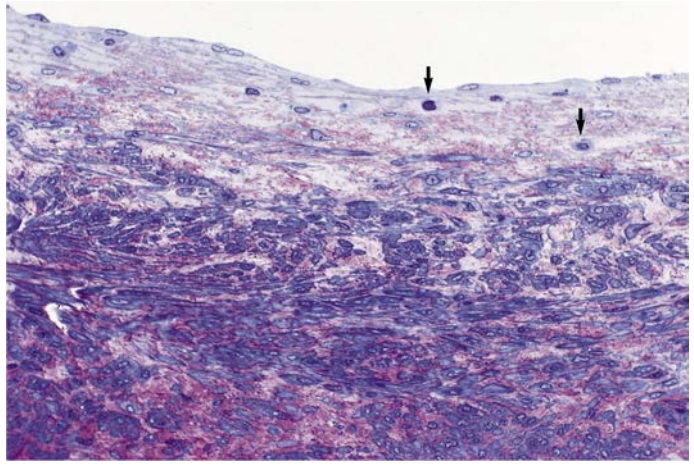
Anatomy



Endothelial dysfunction in atherosclerosis The earliest changes that precede the formation of lesions of atherosclerosis take place in the endothelium. These changes include increased endothelial permeability to lipoproteins and other plasma constituents, which is mediated by nitric oxide, prostacyclin, platelet-derived frowth factor, angiotensin II, and enothelin; up-regulation of leukocyte adhesion molecules, including L-selectin, integrins, and platelet-endothelial-cell adhesion molecule-1, and vascular-cell adhesion molecule 1; and migration of leukocytes into the artery wall, which is mediated by oxidized low-density lipoprotein, monocyte chemotactic protein 1, interleukin-8, platelet-derived growth factor, macrophage colony-stimulating factor, and osteopontin.
(Reproduced with permission from: Ross, R, N Engl J Med 1999; 340:115.

Factors that Cause and Interventions that Improve Endothelial Dysfunction

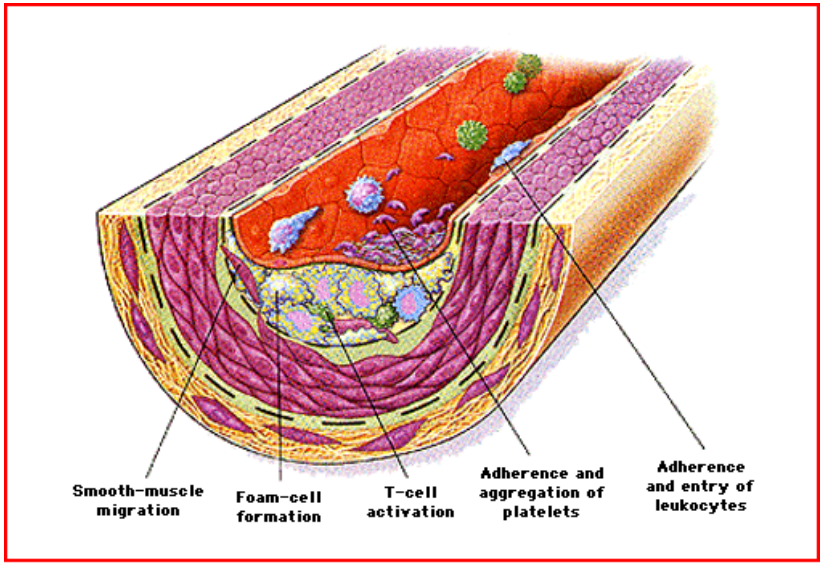
Factors associated with endothelial dysfunction	Interventions that improve endothelial function
Increased age	L-arginine
Male sex	Estrogen
Family history of CHD	Antioxidants
Smoking	Smoking cessation
Increased serum cholesterol	Cholesterol lowering
Low serum HDL-cholesterol	ACE inhibitors
Hypertension	Exercise
Increased serum homocysteine	Homocysteine lowering
Diabetes mellitus	
Obesity	
High-fat meal	



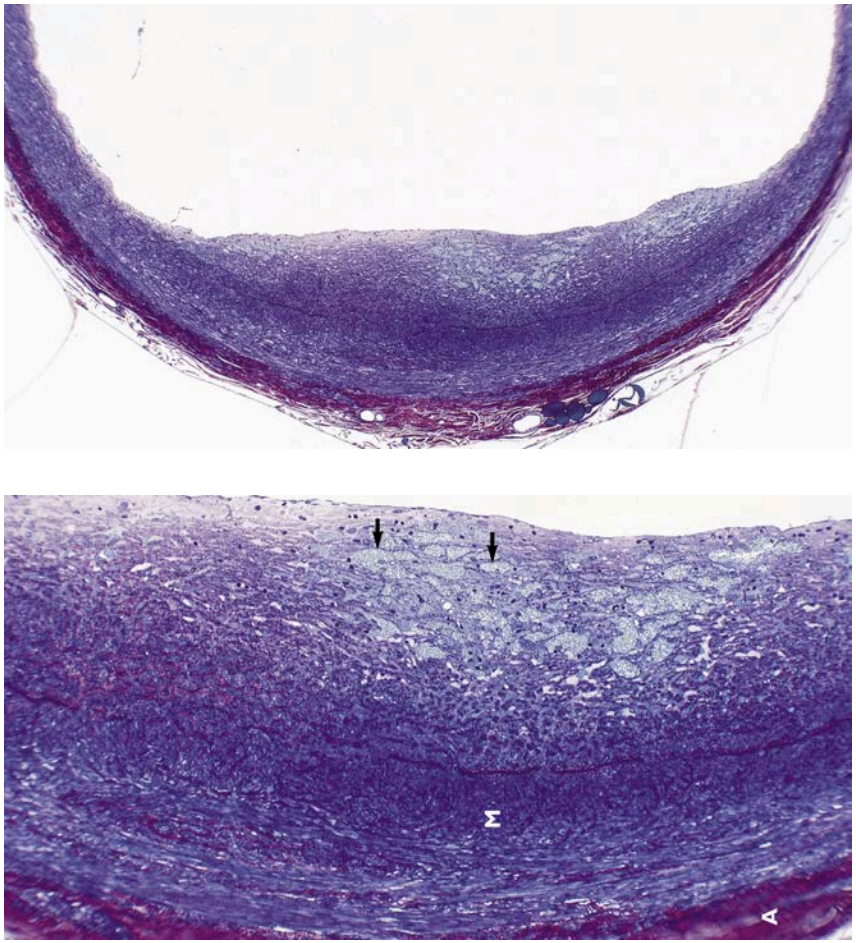
The Vessel Wall is an Organ –
Endothelial barrier is not inert

Stary, H. C. (2003). Atlas of atherosclerosis: progression and regression. Boca Raton, Parthenon Pub. Group.

Pathophysiology of Atherosclerosis



Fatty-streak formation in atherosclerosis Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes. Later they are joined by various numbers of smooth-muscle cells. The steps involved in this process include smooth-muscle migration, which is stimulated by platelet-derived growth factor, fibroblast growth factor 2, and transforming growth factor β; T-cell activation, which is mediated by tumor necrosis factor (TNF) alpha, interleukin (IL)-2, and granulocyte-macrophage colony-stimulating factor; foam-cell formation, which is mediated by oxidized low-density lipoprotein, macrophage colony-stimulating factor, TNF alpha, and IL-1; and platelet adherence and aggregation, which are stimulated by integrins, P-selectin, fibrin, thromboxane A2, tissue factor, and the factors described as responsible for the adherence and migration of leukocytes. (Reproduced with permission from: Ross, R, N Engl J Med 1999; 340:115. Copyright © 1999 Massachusetts Medical Society. All rights reserved.)



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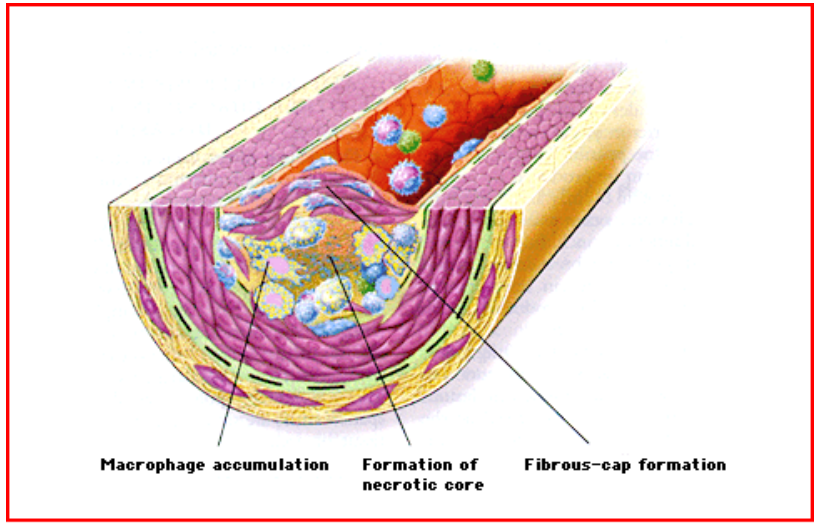
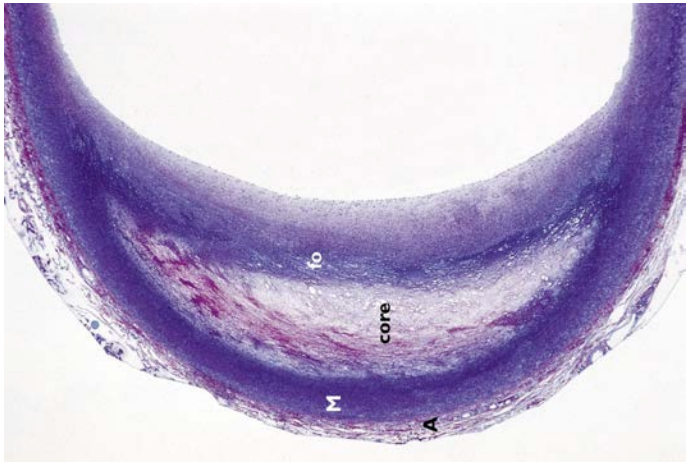
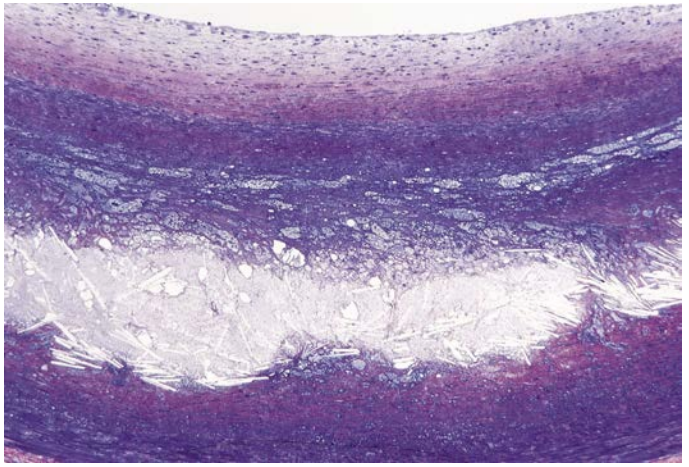
Pathophysiology of Atherosclerosis



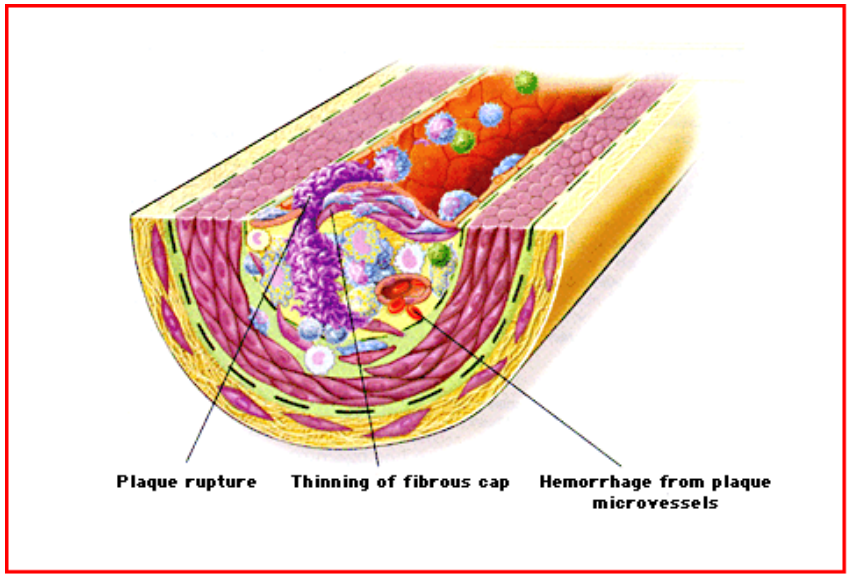
Formation of an advanced, complicated lesion of atherosclerosis
As fatty streaks progress to intermediate and advnaces lesions, they tend to form a fibrous plaque that wall off the lesion from the lumen. This represents a type of healing or fibrous response to the injury. The fibrous plaque covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core. These lesions expand at their shoulders by means of continued leukocyte adhesion and entry, principally due to macrophage colony-stimulating factor, monocyte chemotactic protein 1, and oxidized low density lipoprotein. The necrotic core results from apoptosis and necrosis, increased proteolytic activity, and lipid accumulation. The fibrous cap forms as a result of increased activity of platelet derived growth factor, transforming growth factor ß, interleukin-1, tumor necrosis factor alpha, and osteoprotin and of decreased connective tissue degradation. (Reproduced with permission from Ross, R. N Engl J Med 1999; 340:115. Copyright © 1999 Massachusetts Medical Society. All rights reserved.).



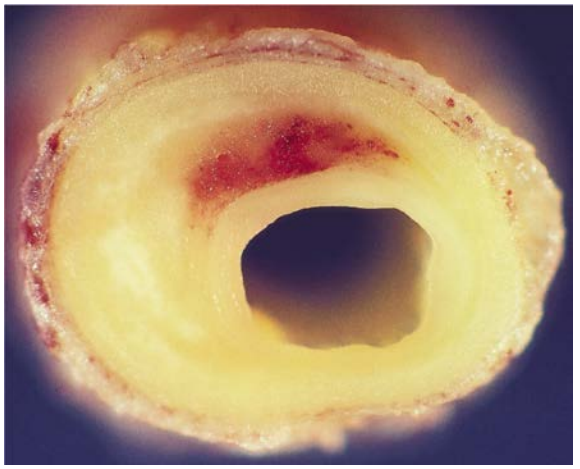


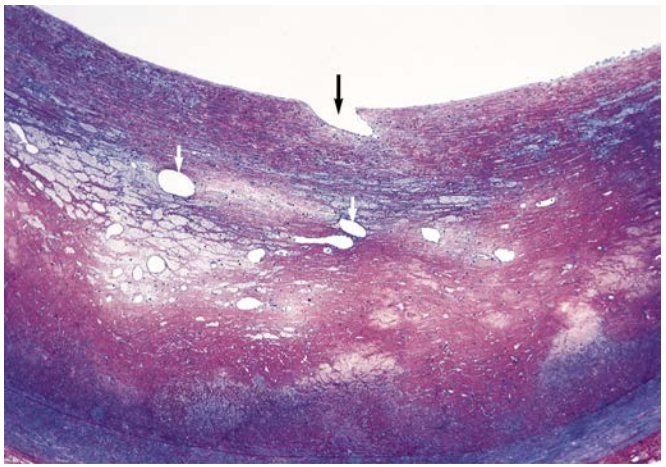
Stary, H. C. (2003). Atlas of atherosclerosis: progression and regression. Boca Raton, Parthenon Pub. Group

Pathophysiology of Atherosclerosis



Unstable fibrous atherosclerotic plaque Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis. It usually occurs at sites of thinning of the fibrous plaque that covers the advanced lesion. Thinning of the fibrous cap is probably due to the continuing influx and activation of macrophages, which release metalloproteinases and other proteolytic enzymes from these sites. These enzymes cause degradation of the matrix, which can lead to hemorrhage from the vaso vasorum or from the lumen of the artery and can result in thrombus formation and occlusion of the artery. (Reproduced with permission from: Ross, R, N Engl J Med 1999; 340:115. Copyright © 1999 Massachusetts Medical Society. All rights reserved.)





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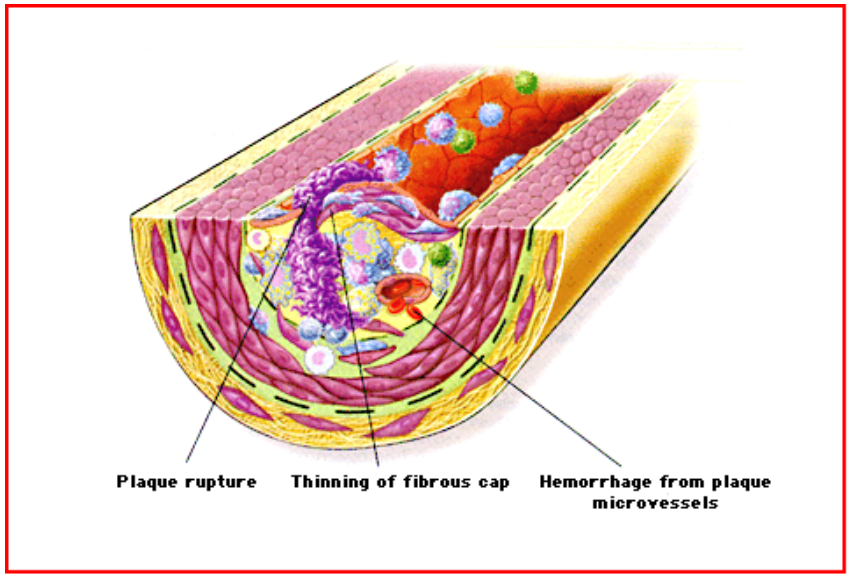
Pathophysiology of Atherosclerosis



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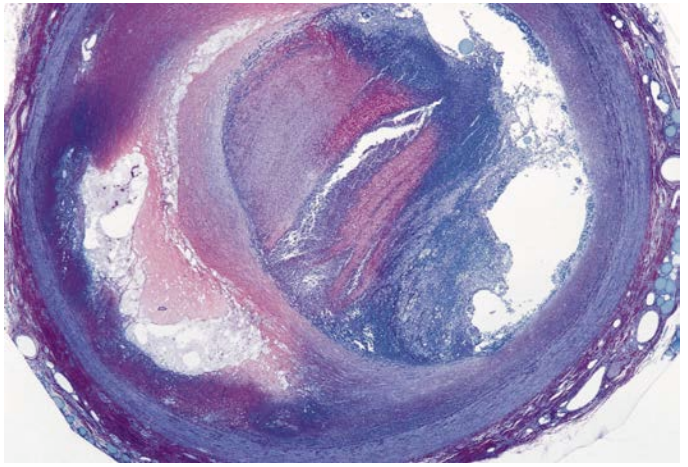

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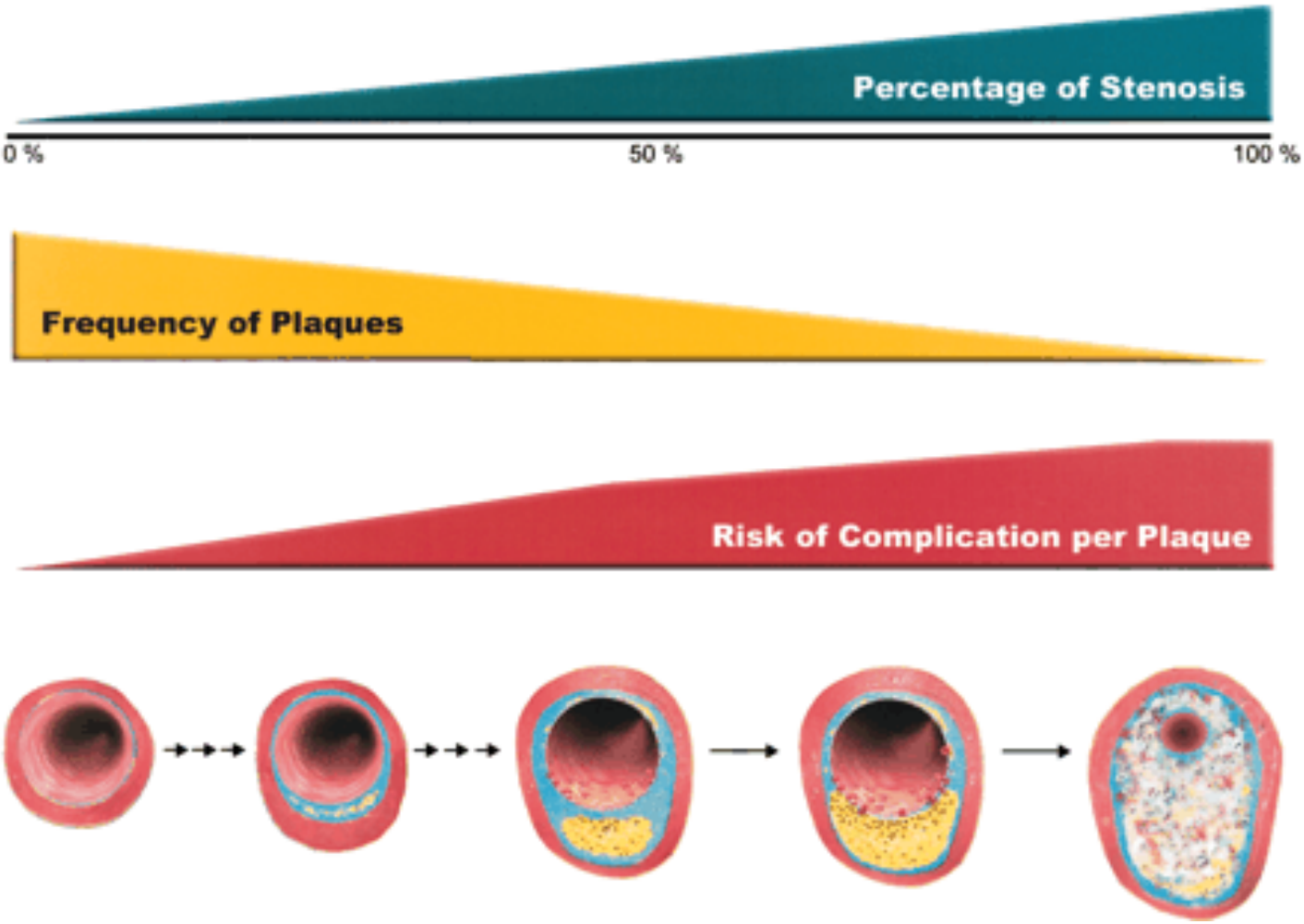
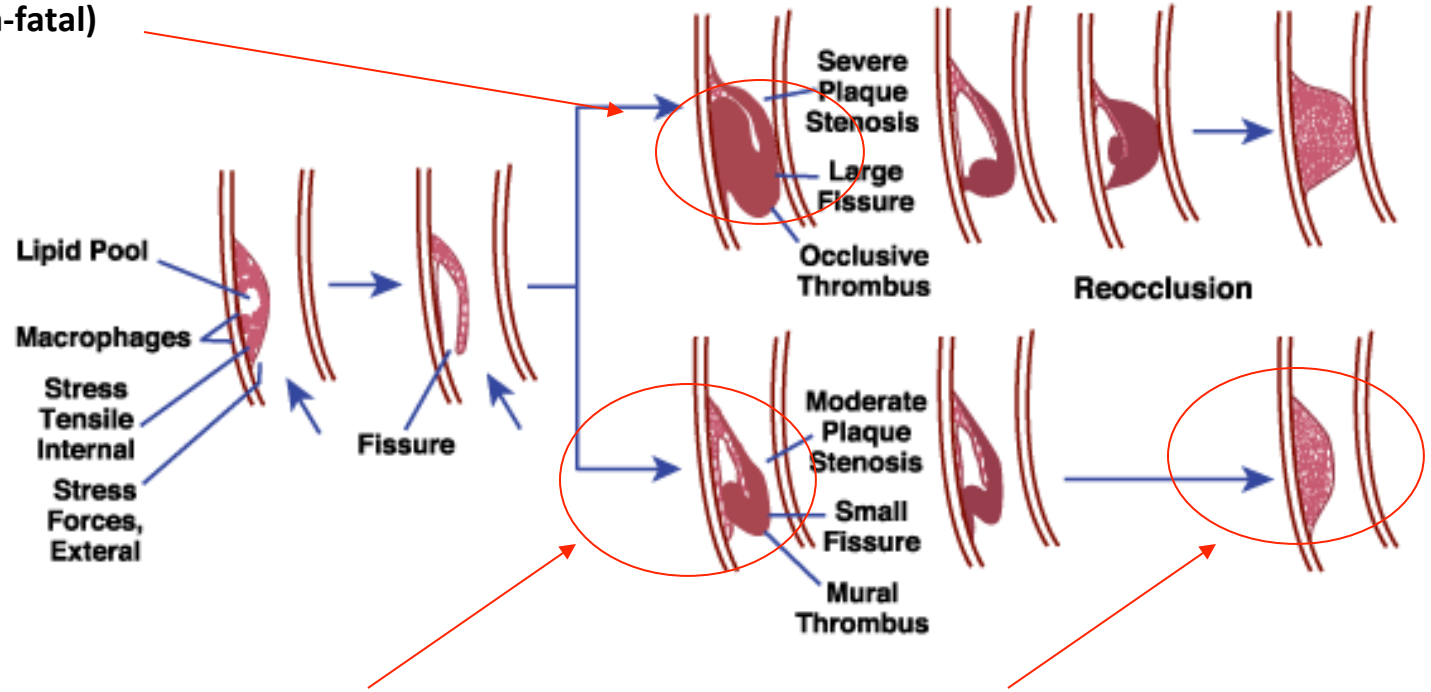


Circulation. 2003;108:1664

Evolution of Coronary Atherosclerosis

Growing Plaque					
Early Lesion		Complicated Plaque			Old Fibrotic Occlusion
Stary I-IV	Stary V	Disruption, Mural Thrombus	Disruption, Occlusive Thrombus	No Disruption, Occlusive Thrombus	

M. I. (fatal, non-fatal)



Unstable angina OR asymptomatic

Stable (exertional) angina

Fuster V, Badimon L, Badimon JJ, et al. The pathogenesis of coronary artery disease and the acute coronary syndromes. N Engl J Med 1992;326:242-50

Complications of heart disease

- **Angina** → not enough blood flow to a part of heart causes chest pain typically during exercise
- **Heart attacks (myocardial infarction)**
  - Death of a part of the heart if blocked artery not opened up within 6 hours
  - If part of heart involved is large death from very very weak heart
  - Arrythmias during a heart attack (short circuits) → death
- **Heart failure**
  - shortness of breath (blood backs up into lungs)
  - Fatigue (blood doesn't go forward enough)
  - weak heart become big and floppy and also predisposes to lethal arrythmias from short circuits
    - Implantable defibrillators
    - Medicines to prevent heart attacks and unload the heart
    - Heart transplant

ESTABLISHED RISK FACTORS FOR CHD

- Age
- Sex
- Cigarette smoking
- High cholesterol
- High blood pressure
- Diabetes mellitus
- Family history
- Obesity (BMI)
- Lack of Exercise

Wilson, P., Culleton BF, **Overview of the risk factors for cardiovascular disease**, Up to Date 2004

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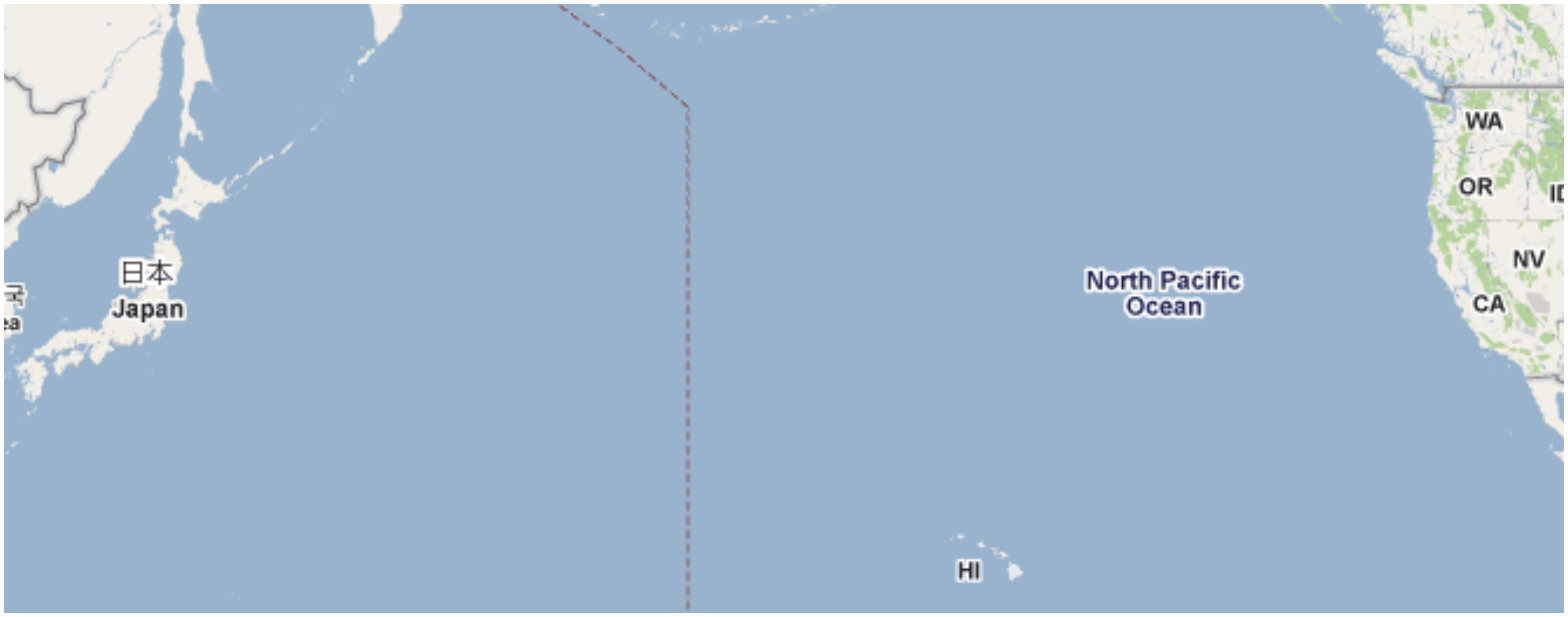
Early studies (1960s) used to estimate relative effect of genetics vs. environment - Migrant studies

TABLE 9-1 The Ni-Hon-San Study Design

Characteristic	Japan	Hawaii	California
Residence	Hiroshima, Nagasaki	Honolulu	San Francisco
Initial exam date	1965-1966	1965-1968	1969-1970
Number examined	2,141	8,006	1,844
Response rate (%)	80	72	68
Mean age (years)	56.2	54.4	52.8



Robertson TL, Kato H, Gordon T, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. Coronary heart disease risk factors in Japan and Hawaii. Am J Cardiol 1977;39:244-9.

Migrant studies - The Ni-Hon-San

TABLE 9-4 Age-Adjusted Prevalence and Incidence of Coronary Heart Disease Among Japanese Men by Place

Disease Measure	Japan	Hawaii	California
Prevalence of myocardial infarction by ECG/1,000	25.0	35.0	45.0
2-year incidence of definite CHD/1,000 person-years	1.4	3.0	4.3
12-year incidence of fatal CHD/1,000 person-years	1.0	1.4	—

Note: ECG = electrocardiogram; CHD = coronary heart disease.

TABLE 9-2 Age-Adjusted Means and Percentages of Selected Variables at Baseline Examination for the Ni-Hon-San Study

Variable	Japan	Hawaii	California
Body mass index (kg/m^2)	21	24[a]	24[a]
Systolic blood pressure (mm Hg)	134	134	138[a]
Diastolic blood pressure (mm Hg)	84	82	88[a]
Serum cholesterol (mg/dL)	186	218[a]	225[a]
Serum glucose (mg/dL)	145	162[a]	160[a]
Current smokers (%)	76	44[a]	35[a]

[a] Significantly different from Japan ($p < .05$).

Robertson TL, Kato H, Gordon T, et al. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. Coronary heart disease risk factors in Japan and Hawaii. Am J Cardiol 1977;39:244-9.

The 1970s & 1980s
Familial aggregation as surrogate for genetics

Summary of Analyses of prospective studies of Family History of CAD and incidence of CAD

Year	Study	Subjects	F/U (yrs)	Definititon of Family History	Disease Endpoint	Unadjusted RR	Adjusted RR	Covariates
1975	Western Collaborative study	3154 men (age 39-59)	8.5	parental history of angina or MI (fatal or non-fatal)	symptomatic MI	2.02**	1.67	education, Type A personality, TC, LDL/HDL
					symptomatic angina	2.55*	2.05	
					symptomatic CAD	2.17***	1.81**	
1984	Rancho Bernardo, CA residents	1774 men, 2240 women (age 40-79)	9	Heart attack in any first degree relative	CVD death- Men	1.52*	1.5*	TC, obesity, smoking, DM
					CVD death-Men< 60		7.6	
					CAD death- Men	1.59*	1.56*	
					CAD death- Men<60		6.8	
					CVD death-women	0.78	0.82	
					CVD death-women	0.94	0.87	
1986	Nurses Health Study	117156 women (age 30-55)	4	Parental MI at age ≤ 60	Non Fatal MI	2.8(2.0-4.1)	2.4(2.0-3.0)	HTN, DM, TC, smoking, obesity, OC, menopause, HRT (not LDL, HDL, EtOh)
				Parental MI at age ≤ 60	Fatal CAD	5(2.7-9.2)	4.9(3.3-7.1)	
				Parental MI at age > 60	Non Fatal MI		1(0.5-1.7)	
1988	British Regional Heart Study	7735 men (age 40-59)	6.2	Parental death from heart disease	Non Fatal MI or fatal MI	Father died: 2.52(1.7-3.8)	2.11(1.4-3.3)	smoking, BP, TC, HDL
						Mother died 1.56(1.1-2.3)	1.32(0.9-1.9)	
1988	The Utah Cardiovascular Genetic Research	1196 men/women (aged > 20)	2	No. of relatives with CAD < 55	CAD		1.59(1.2-2.1)	age, gender, TC, HDL, DM, smoking, BMI
1989	Framingham	3933 men/women (aged 28-62)	28	Parental CAD death at age <65	CAD-Men	1.2(0.9-1.7)	1.3 (0.8-2.0)	age, gender, SBP, TC, IR, smoking, relative body weight, LVH
					CAD-Women	1.3(1.0-1.9)	1.6 (1.2-2.3)	
					CAD-Combined	1.2 (1.0-1.5)	1.4 (1.1-1.8)	
1991	Health Professional	45317 men health professionals	2	both parents with MI age < 70	Non Fatal MI or fatal MI	2.0(1.3-3.2)	1.8(1.1-3.1)	BMI, DM, HTN, TC, smoking, diet, EtOH, profession
				mother < 50			3.7	
				father < 50			2.3	

Problem with familial aggregation

- Confounding by shared "environmental factors" → "traditional risk factors"
- The *ideal* family study
  - large adoption study tracking in both parents and in offspring
    - incident CAD events
    - Traditional risk factors of CAD in offspring and in parents
    - Random allocation of offspring to new parents
  - Uncouples the shared environment from the shared genetics
  - Issues with feasibility and adoption bias
- Twin studies an alternative feasible and informative design

Twin studies

- Assumption: environmental influences equal in both types of twins (monozygotic/dizygotic)
- Concordance Rate (CR) as an estimate of genetic effects
  - CR = concordant pairs / concordant pairs + discordant pairs
  - Strictly genetic trait: MZ = 100% , DZ = 25-50%
  - Complex trait: low concordance rate
  - heritability (h^2) using twin correlations in MZ and DZ twins
    - $h^2=2(r(MZ)-r(DZ))$

Concordance rates and

Heritability Based on Twin Data

Heritability estimates the contribution of genetic elements to the phenotype.

	MZ twin	DZ twin	Heritability
High Blood Pressure	0.6 - 0.8	0.3 - 0.5	0.60
Asthma	0.12 - 0.89	0 - 0.5	0.72 - 0.8
Type I Diabetes	0.25 - 0.35	0.03 - 0.05	0.72
Type 2 Diabetes	0.50	0.37	0.26
Rheumatoid Arthritis	0.15	0.04	0.32

Genetic susceptibility to death from coronary heart disease in two studies of twins

	MZ concordance rate	DZ concordance rate	Heritability Swedish cohort	Heritability Danish cohort
MALES	0.34	0.23	0.58	0.53
FEMALES	0.24	0.16	0.38	0.58

Marenberg, M. E., N. Risch, et al. (1994). N Engl J Med **330**(15): 1041-6

Wienke A, Holm NV, Skytthe A, Yashin AI. The heritability of mortality due to heart diseases: a correlated frailty model applied to Danish twins. Twin Res 2001;4:266-74.

Genetic susceptibility to death from coronary heart disease in a study of twins
Discrete time survival analysis adjusting for risk factors

Summary of Analyses of prospective studies of Family History of CAD and incidence of CAD

Year	Study	Subjects	F/U (yrs)	Definititon of Family History	Disease Endpoint	Unadjusted RR	Adjusted RR	Covariates
1994	Swedish Twin Registry	5964 DZ males (36-75 yrs)	26	Twin dies of CAD < 55 yrs (ICD)	CAD death (ICD)	4.3 (1.2-10.6)	3.8 (1.4-10.5)	smoking, BMI, DM, HTN, education, marital status
		3298 MZ males (36-75 yrs)				13.4 (5.1-35.1)	8.1 (2.7-24.5)	
		7750 DZ females (36-75 yrs)		Twin dies of CAD age < 65 yrs (ICD)		2.2 (1.7-3.9)	2.6 (1-7.1)	
		4012 MZ females (36-75 yrs)				14.9 (7.5-29.6)	15.0 (7.1-31.9)	

About half of the heritability of CAD is a consequence of heritability of traditional risk factors (adiposity, lipids, blood pressure, diabetes)

Marenberg, M. E., N. Risch, et al. (1994). N Engl J Med **330**(15): 1041-6

CAD is a complex trait, even if no other risk factors are ever discovered

TABLE 1 Genetic and environmental risk factors for CVD[1]

Risk factors with a significant genetic component (heritability[2])
Myocardial infarction (25% to 60%)
Stroke
Total cholesterol (40% to 60%)
HDL-cholesterol (45% to 75%)
Total triglycerides (40% to 80%)
Body mass index (25% to 60%)
Systolic blood pressure (50% to 70%)
Diastolic blood pressure (50% to 65%)
Lp(a) levels (90%)
Homocysteine levels (45%)
Type 2 diabetes (40% to 80%)
Fibrinogen (20% to 50%)
C-reactive protein
Gender
Age
Environmental risk factors
Smoking
Diet
Exercise
Infection
Fetal environment
Air pollution (particulates)

[1]From (51).

[2]Heritability estimates, in most cases based on multiple studies, are taken from Lusis (52), King et al. (45), Jee et al. (39), and Tournier-Lasserve (95).

Lusis et al. Genetics of Atherosclerosis. Annu. Rev. Genomics Hum. Genet. 2004. 5:189–218

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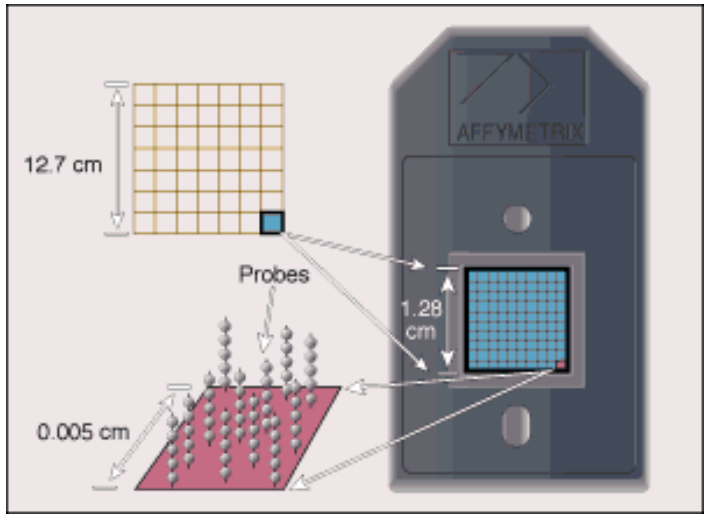
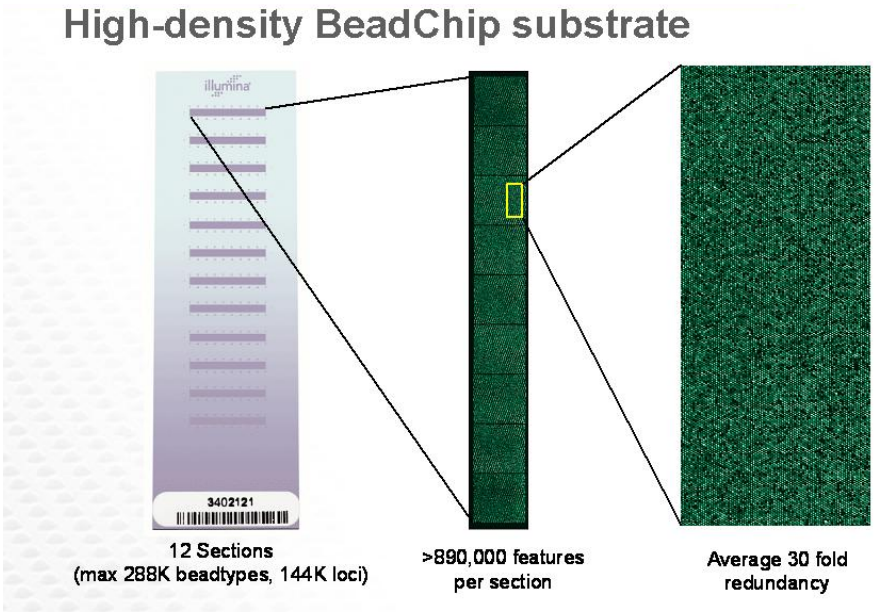
Gene chip "genome wide" genotyping systems Test Common Disease-Common Variant Hypothesis In **Genome Wide Association Study (GWAS)**

Affymetrix



Illumina



$1.5x10^6$ Quasi-Random SNPs

$1x10^6$ low LD SNPs

A significant proportion of common SNPs can be captured
Because of LD inform about many others
Problem: overlap of SNP on each platform minimal

GWAS Meta analysis

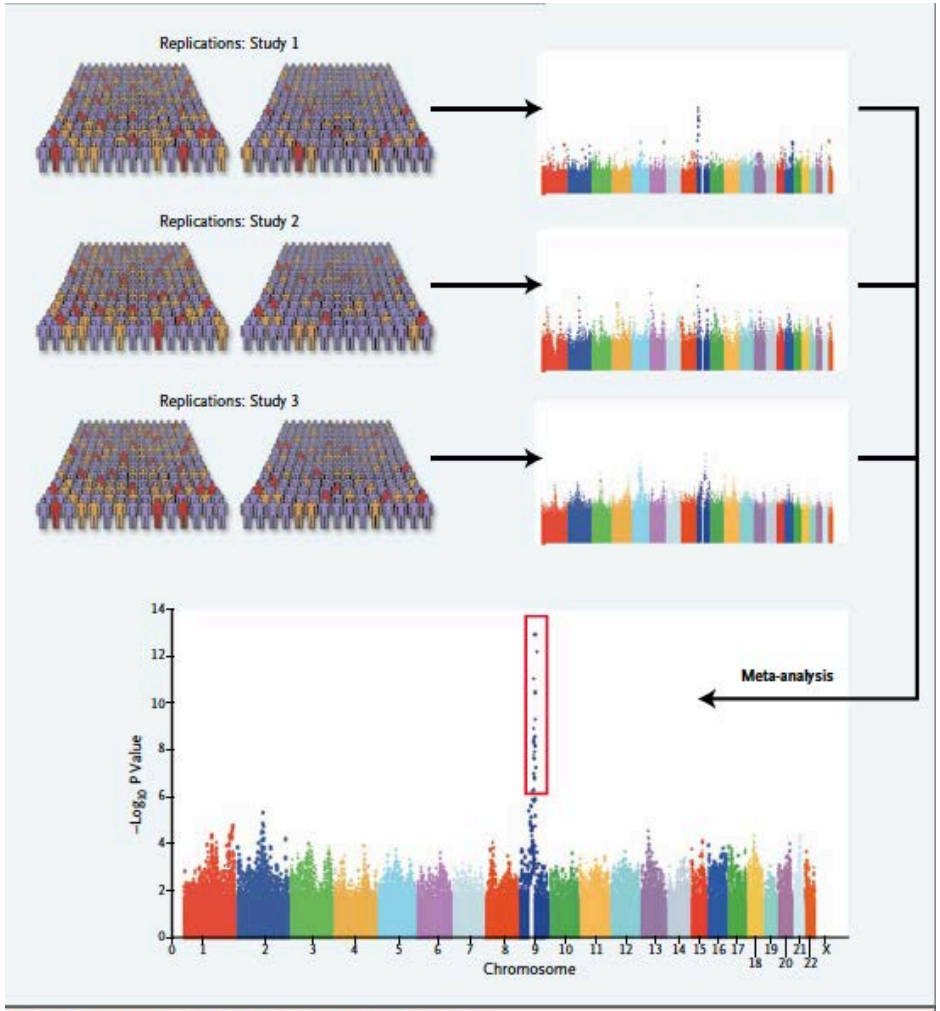


Figure 2. Meta-Analysis of Genomewide Association Studies.

Manolio, T. A. (2010). "Genomewide association studies and assessment of the risk of disease." N Engl J Med **363(2): 166-176.**

The first "real" CAD signal

Sciencexpress Report

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] Anne Tybjaerg-Hansen,[5] Aaron R. Folsom,[6] Eric Boerwinkle,[7] Helen H. Hobbs,[2,9] Jonathan C. Cohen[2,8†]

Sciencexpress Report

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 Blondal,[1] Aslaug Jonasdottir,[1] Adalbjorg Jonasdottir,[1] Asgeir Sigurdsson,[1] Adam Baker,[1] Arnar Palsson,[1] Gisli Masson,[1] Daniel 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] Svati H. Shah,[5] Arshed A. Quyyumi,[3] Jeffrey R. Gulcher,[1] Gudmundur Thorgeirsson,[3] Unnur Thorsteinsdottir,[1] Augustine Kong,[1†] Kari Stefansson[1†]

[1]deCODE genetics Inc, Reykjavik, Iceland. [2]National University Hospital, Reykjavik, Iceland. [3]Emory University School of Medicine, Atlanta, GA 30322, USA. [4]University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA. [5]Duke University School of Medicine, Durham, NC 27710, USA.

Loci associated with AMI/CAD

(as of Feb, 2011)

Unique locus	Chr	SNP	Risk allele frequency (%)	Odds ratio (95% CI) per risk allele	Gene(s) of interest within or near associated interval	Associated with low-density lipoprotein cholesterol or lipoprotein (a)?
1	9p21	rs4977574	56	1.29 (1.25–1.34)	*CDKN2A-CDKN2B-ANRIL*	—
2	1p13	rs646776	81	1.19 (1.13–1.26)	*CELSR2-PSRC1-SORT1*	Yes
3	21q22	rs9982601	13	1.20 (1.14–1.27)	*SLC5A3-MRPS6-KCNE2*	—
4	1q41	rs17465637	72	1.14 (1.10–1.19)	*MIA3*	—
5	10q11	rs1746048	84	1.17 (1.11–1.24)	*CXCL12*	—
6	6p24	rs12526453	65	1.12 (1.08–1.17)	*PHACTR1*	—
7	19p13	rs1122608	75	1.15 (1.10–1.20)	*LDLR*	Yes
8	2q33	rs6725887	14	1.17 (1.11–1.23)	*WDR12*	—
9	1p32	rs11206510	81	1.15 (1.10–1.21)	*PCSK9*	Yes
10	12q24	rs2259816	37	1.08 (1.05–1.11)	*HNF1A*	Yes
11	12q24	rs3184504	40	1.13 (1.08–1.18)	*SH2B3*	—
12	3q22	rs9818870	15	1.15 (1.11–1.19)	*MRAS*	—
13	6q26-6q27	rs3798220	2	1.47 (1.35–1.60)	*LPA*	Yes
		rs10455872	7	1.68 (1.43–1.98)	*LPA*	Yes

Musunuru K, Kathiresan S. Genetics of coronary artery disease. Annu Rev Genomics Hum Genet 2010;11:91-108.

18 new loci

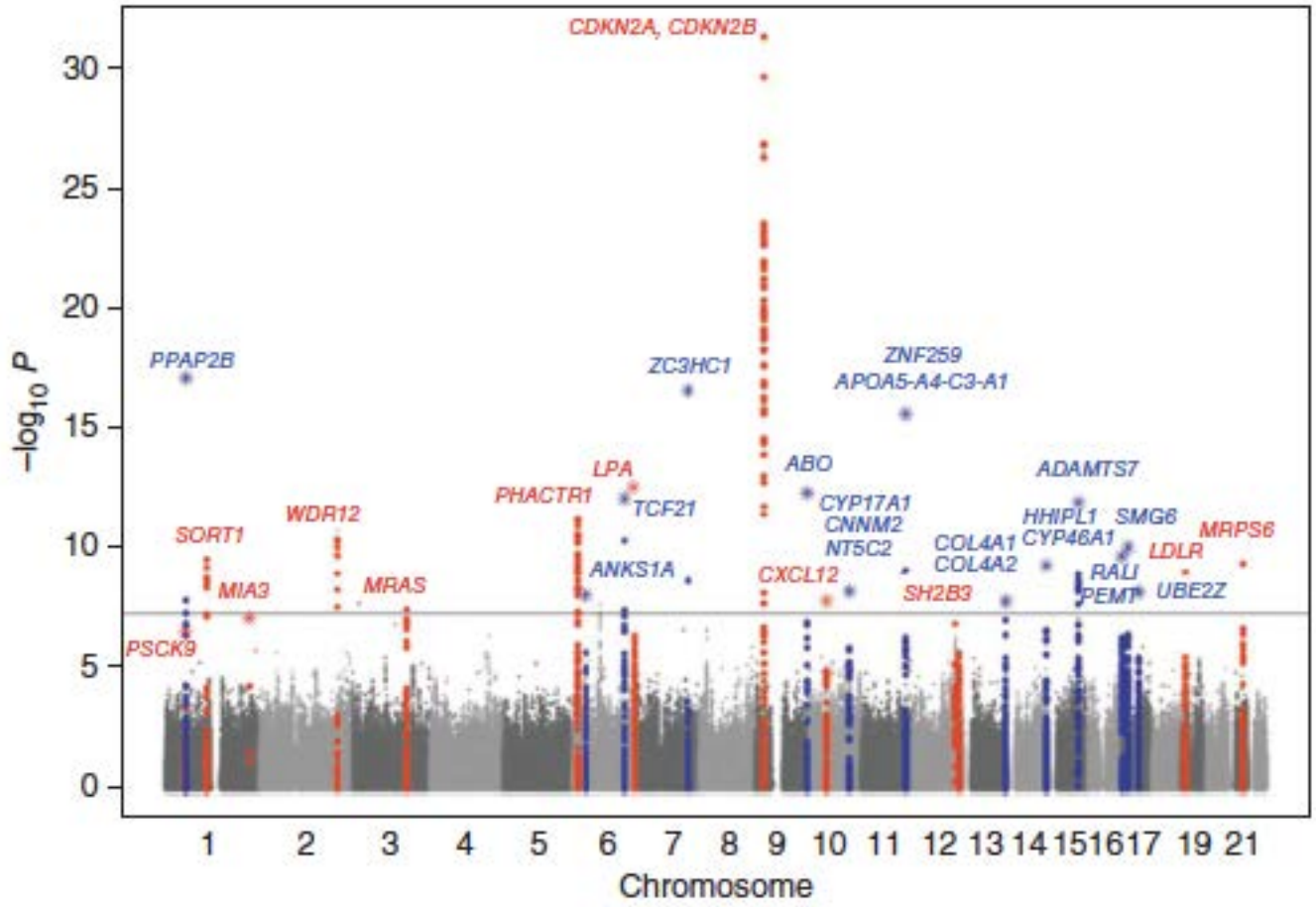
CARDIoGRAM and C4D consortia (2 reports)

chrom	gene region	Allele Freq	OR	P value	SNP*	Reference
1p32.2	*PPAP2B*	*0.91*	1.17 (1.13–1.22)	3.81 × 10−19	rs17114036	cardiogram
6p21.31	*ANKS1A*	*0.75*	1.07 (1.05–1.10)	1.36 × 10−8	rs17609940	cardiogram
6q23.2	*TCF21*	*0.62*	1.08 (1.06–1.10)	1.07 × 10−12	rs12190287	cardiogram
7q32.2	*ZC3HC1*	*0.62*	1.09 (1.07–1.12)	9.18 × 10−18	rs11556924	cardiogram
9q.34.2	*ABO*	*0.21*	1.10 (1.07–1.13)	4.08 × 10−14	rs579459	cardiogram
10q24.32	*CYP17A1, CNNM2, NT5C2*	*0.89*	1.12 (1.08–1.16)	1.03 × 10−9	rs12413409	cardiogram
11q23.3	*ZNF259, APOA5-A4-C3-A1*	*0.13*	1.13 (1.10–1.16)	1.02 × 10−17	rs964184	cardiogram
13q34	*COL4A1, COL4A2*	*0.44*	1.07 (1.05–1.09)	3.84 × 10−9	rs4773144	cardiogram
14q32.2	*HHIPL1*	*0.43*	1.07 (1.05–1.10)	1.14 × 10−10	rs2895811	cardiogram
15q25.1	*ADAMTS7*	*0.57*	1.08 (1.06–1.10)	1.07 × 10−12	rs3825807	cardiogram
17p13.3	*SMG6, SRR*	*0.37*	1.07 (1.05–1.09)	1.15 × 10−9	rs216172	cardiogram
17p11.2	*RASD1, SMCR3, PEMT*	*0.56*	1.07 (1.05–1.09)	4.45 × 10−10	rs12936587	cardiogram
17q21.32	*UBE2Z, GIP, ATP5G1, SNF8*	*0.53*	1.06 (1.04–1.08)	1.81 × 10−8	rs46522	cardiogram
10	*LIPA*	*0.34*	1.09 (1.07–1.12)	2.76 x 10-13	rs1412444	C4D
11	*PDGFD*	*0.29*	1.07 (1.04–1.09)	2.41 x 10-9	rs974819	C4D
15	*ADAMTS7-MORF4L1*	*0.6*	1.07 (1.05–1.10)	3.71 x 10-9	rs4380028	C4D
7q22	None	0.75	1.08 (1.05–1.11)	3.12 x 10-8	rs10953541	C4D
10	*KIAA1462*	*0.42*	1.07 (1.04–1.09)	3.87 x 10-8	rs2505083	C4D

Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011

Peden JF, Hopewell JC, Saleheen D, et al. A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease. Nat Genet 2011.

Manhattan Plot
CARDIoGRAM consortium



~3-4% of variance
~7% of genetic Variance (assuming heritability of 50%)

Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011

CARDIoGRAM+C4D together
13 additional loci - unpublished

Table 2. Additional loci reaching genome wide significance

NP	Chr	Nearest Gene(s)	Effect/Non Effect allele (frequency)	Stage 1 (18,014 cases/ 40,925 controls)[1] OR	Stage 1 *P*	Stage 2 (40,365 cases/ 63,714 controls) OR	Stage 2 *P*	Combined (Stage 1,2) *P*	Stage 3 (5,055 cases/ 5,617 controls) OR	Stage 3 *P*	Combined (Stage 1,2,3) *P*	Biological relevance
						Novel						
15625	1	IL6R	T/C (0.47)	1.06	4.84E-05	1.04	3.46E-05	**3.55E-08**	1.09	1.58E-03	**3.64E-10**	2
5135	2	APOB	C/T (0.83)	1.07	8.63E-04	1.08	2.17E-08	**4.80E-10**	1.03	4.02E-01	**2.56E-10**	1
52641	2	ZEB2-AC074093.1	C/T (0.46)	1.06	1.37E-05	1.04	1.27E-04	**3.66E-08**	1.00	9.54E-01	5.30E-08	
51198	2	VAMP5-VAMP8-GGCX	T/C (0.45)	1.06	7.47E-05	1.05	2.57E-06	**4.48E-09**	1.07	1.75E-02	**1.22E-10**	A 1
92387	4	GUCY1A3	G/A (0.81)	1.08	1.04E-05	1.06	1.89E-05	**4.57E-09**	1.13	5.47E-04	**2.65E-11**	1
3909	5	SLC22A4-SLC22A5	G/A (0.14)	1.07	3.24E-03	1.09	2.00E-07	**1.43E-08**	1.11	2.43E-02	**9.62E-10**	A 1
47789	6	KCNK5	T/C (0.76)	1.07	6.07E-05	1.06	1.22E-05	**1.63E-08**	1.01	7.03E-01	**9.81E-09**	3
52120	6	PLG	T/C (0.73)	1.07	1.18E-05	1.06	1.82E-05	**5.00E-09**	1.07	9.58E-02	**4.88E-10**	1
264	8	LPL	G/A (0.86)	1.11	2.99E-07	1.05	7.30E-04	**5.06E-09**	1.06	1.60E-01	**2.88E-09**	1
19428	13	FLT1	A/G (0.32)	1.06	7.88E-05	1.05	5.70E-06	**1.01E-08**	1.10	1.37E-03	**7.32E-11**	1
14846	15	FURIN-FES	A/C (0.44)	1.07	2.37E-05	1.05	7.35E-07	**4.49E-10**	1.04	3.02E-01	**9.33E-11**	A 1
						Previously reported at array wide level of significance ($p < 3 \times 10^{-6}$)						
54029	8	TRIB1	A/T (0.55)	1.06	2.79E-05	1.04	7.75E-05	**4.53E-08**	1.05	8.56E-02	**4.75E-09**	4
14713	2	ABCG5-ABCG8	T/C (0.30)	1.06	2.22E-04	1.06	1.57E-07	**8.72E-10**	0.96	3.56E-01	**2.12E-09**	1
						Novel (Stage 3 replication)						
78406	4	EDNRA	T/C (0.15)	1.10	2.37E-06	1.06	3.54E-03	**1.65E-07**	1.09	2.01E-02	**2.54E-08**	1
23938	7	HDAC9	C/T (0.10)	1.08	6.81E-04	1.07	5.25E-05	**6.49E-07**	1.13	4.09E-02	**4.94E-08**	1

[1]The total sample sizes do not include the CHARGE sample sizes

A cis eQTL in LCL

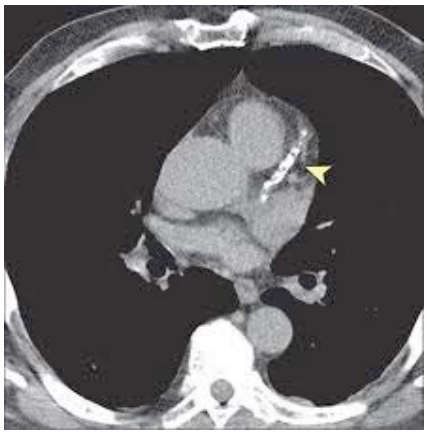
1 Mouse model available with cardiovascular phenotype

2 Mouse model has homeostatic and immune phenotypes

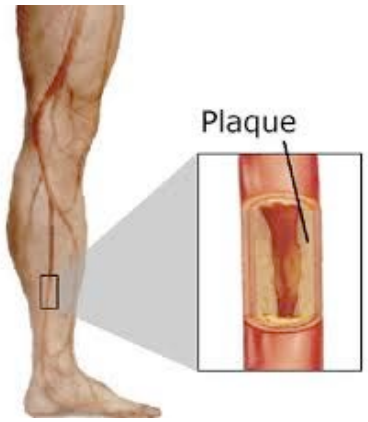
3 Mouse model has respiratory, nervous system, mortality, ageing, growth and renal phenotypes

4 Mouse model has growth and immune phenotypes

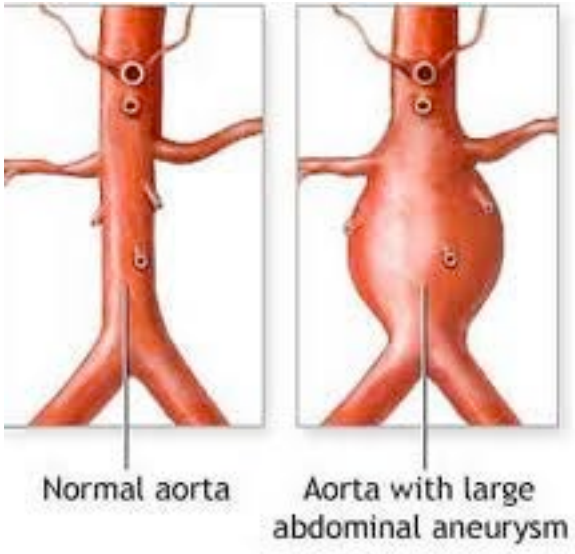
GWAS: Informing Biology even before a single bench-side experiment: **9p21 associations with other outcomes**



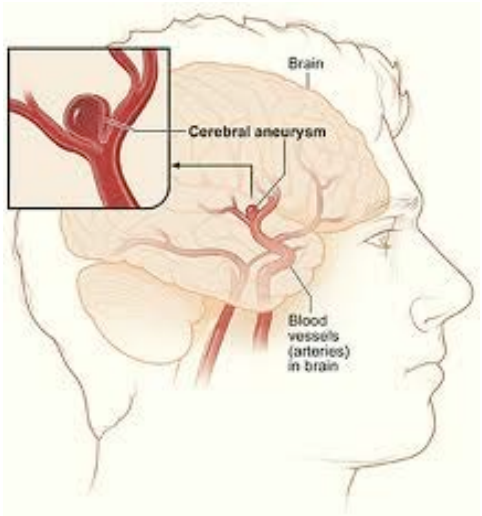
CAC



PAD



AAA



Berry Aneurysm!!

Helgadottir A, Thorleifsson G, Magnusson KP, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet 2008;40:217-24.

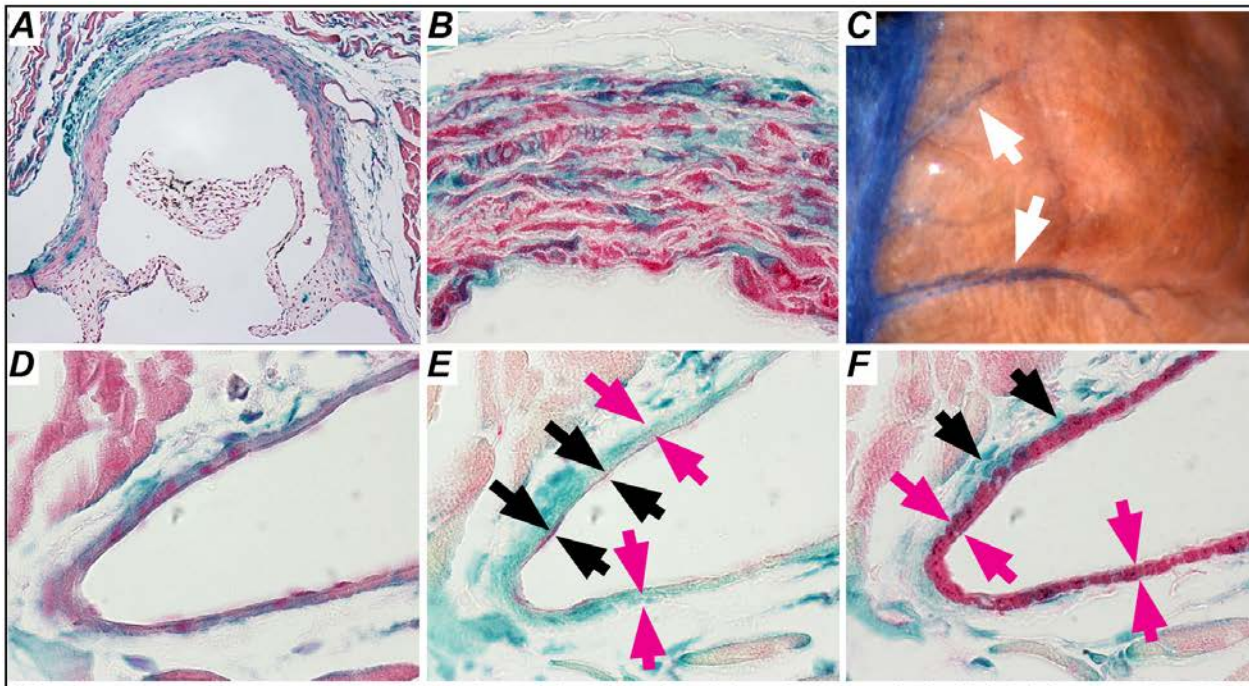
TCF21 (Capsulin)

Cloning of capsulin, a basic helix-loop-helix factor expressed in progenitor cells of the pericardium and the coronary arteries[1]

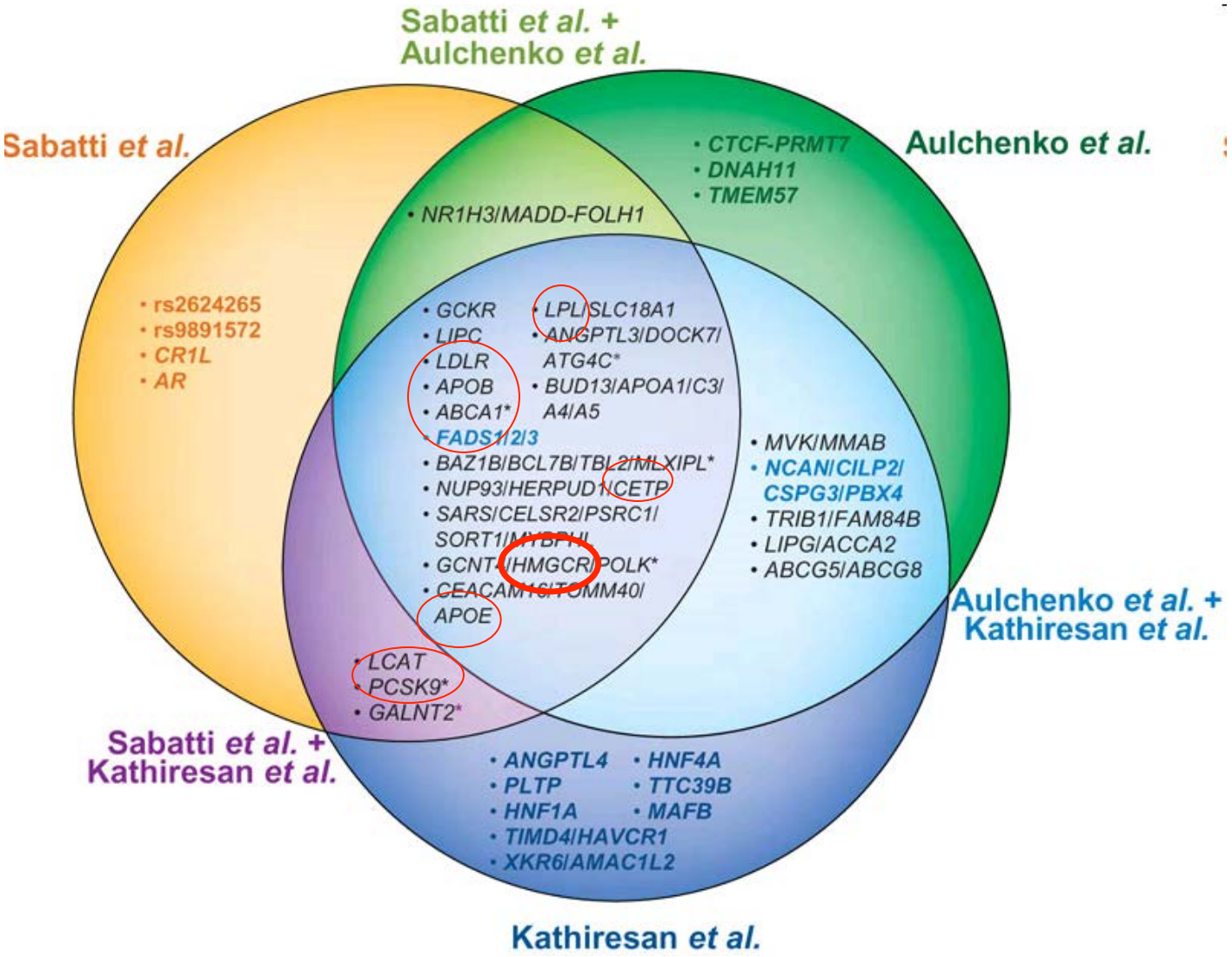
Hiroko Hidai[a], Richard Bardales[b], Richard Goodwin[b], Thomas Quertermous[c,*], Elena E. Quertermous[c]

[a]*Tokyo Women's Medical College, Tokyo 162, Japan*
[b]*Division of Cardiology, Department of Medicine, Vanderbilt University Medical School, Nashville, TN 37232, USA*
[c]*Division of Cardiology, Stanford University School of Medicine, Falk CVRC, 300 Pasteur Drive, Stanford, CA 94305, USA*

Received 2 December 1997; revised version received 3 February 1998; accepted 11 February 1998



GWAS results for Lipids (HDL, LDL, Trig)



S. Kathiresan *et al.*, *Nature genetics* **41**, 56 (Jan, 2009).

Y. S. Aulchenko *et al.*, *Nature genetics* **41**, 47 (Jan, 2009).

C. Sabatti *et al.*, *Nature genetics* **41**, 35 (Jan, 2009)

T. A. Manolio, *Nature genetics* **41**, 5 (Jan, 2009).

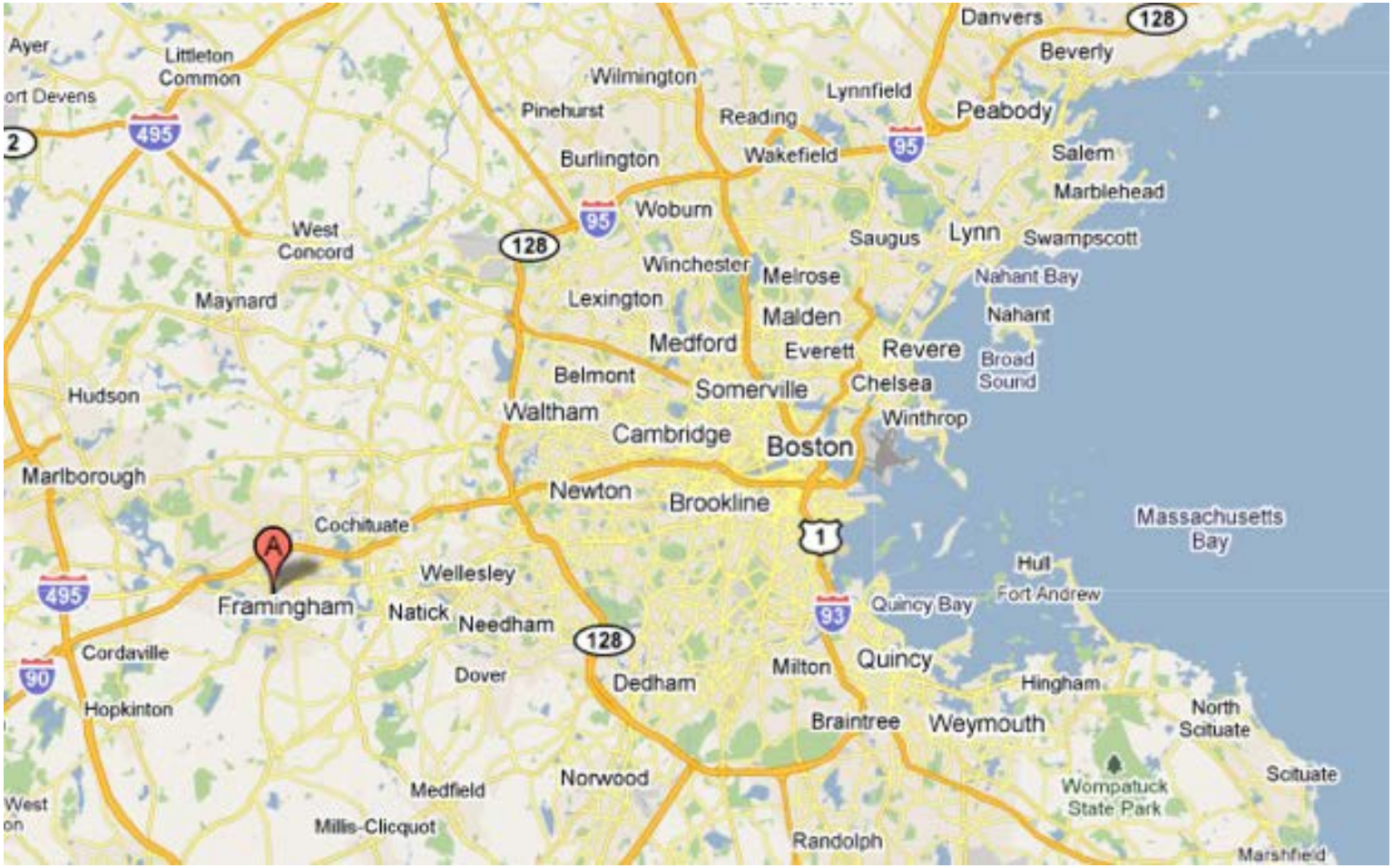
Outline

- Epidemiology of Coronary artery disease
- Pathophysiology of coronary atherosclerosis
  - "CAD", hardening of arteries
- Heritability of CHD and risk factors
- GWAS discoveries
- Current risk prediction for CHD
  - Clinical considerations
- Old and new metrics for assessing improvement in risk prediction algorithms
  - Bringing in genetics
- Future Directions

What we can do to reduce risk in primary prevention

- ++ Lifestyle modification (positive effects on multiple risk factors , e.g. BMI, BP, chol)
- ++ treat high blood pressure to systolic < 140 mmHg and diastolic < 90 mmHG
  - Doesn't really matter what means you use to get there
  - More aggressive reduction (i.e. to < 120/80) with drugs not clearly supported
- ++ treat high LDL, in general the lower the better, the longer the better
- aspirin, perhaps only in people at high risk
- -- routine testing for blockages (treadmills) NOT INDICATED

Framingham Heart Study
Boston U./NHLBI



Framingham original cohort 1948

- 5,209 respondents of a random sample of 2/3 of the adult population of Framingham, Massachusetts in 1948
- Exam 30 for the Original Cohort: May 2008 to Feb 2010
- MAJOR ACCOMPLISHMENT: DISCOVERY OF TRADITIONAL RISK FACTORS FOR CORONARY DISEASE

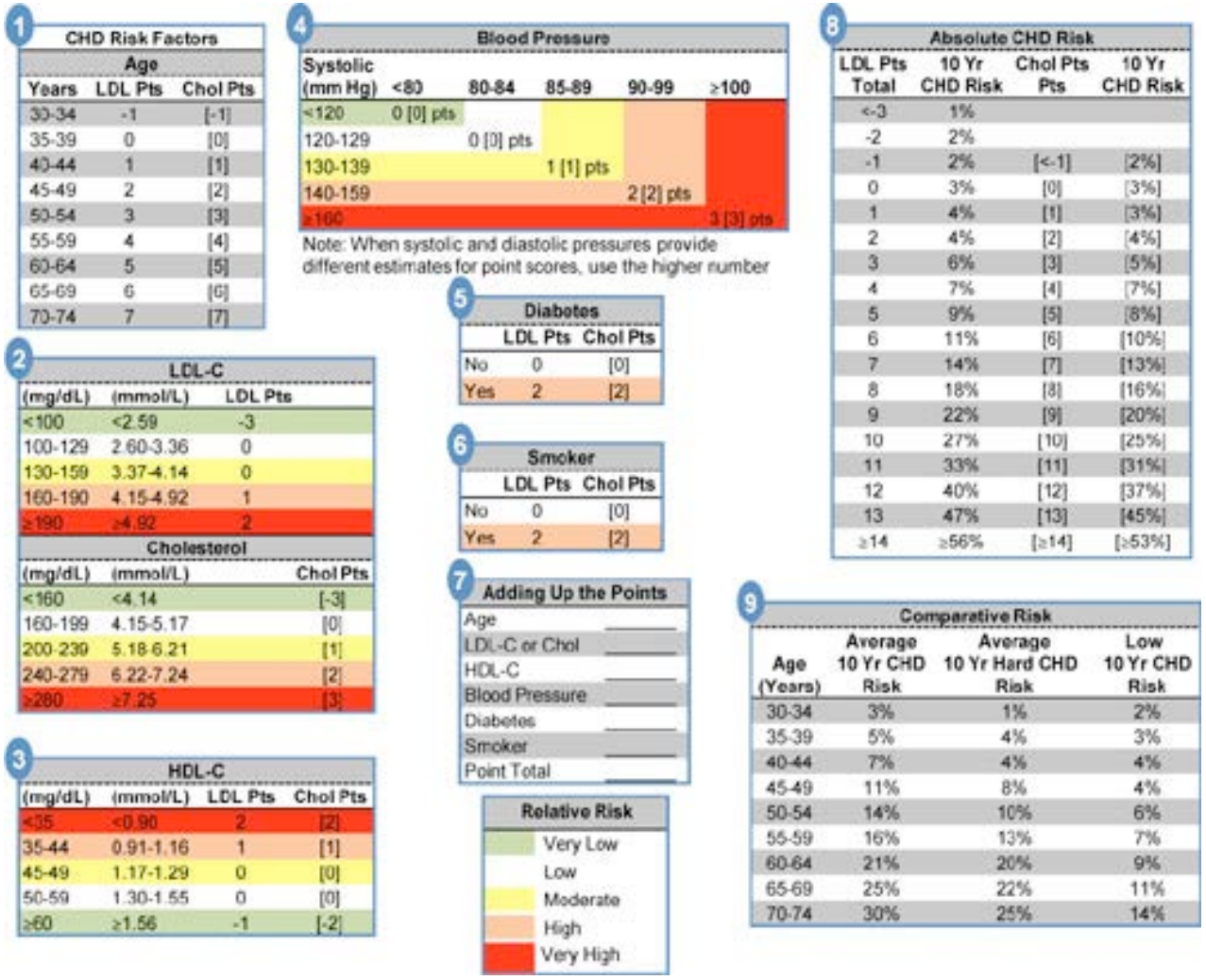
AGE-SEX DISTRIBUTION AT ENTRY (1948)				
Age	**29-39**	**40-49**	**50-62**	**Totals**
Men	835	779	722	2,336
Women	1,042	962	869	2,873
Totals	1,877	1,741	1,591	5,209

Framingham offspring cohort 1971

- 5,124 men and women, consisting of the offspring of the Original Cohort and their spouses was recruited.
- Offspring Exam 9 is scheduled to begin in 2011
- MAJOR ACCOMPLISHMENT: DEVELOPMENT OF THE FRAMINGHAM RISK SCORE

AGE–SEX DISTRIBUTION AT ENTRY (1971)								
Age	**<10**	**10–19**	**20–29**	**30–39**	**40–49**	**50–59**	**60–70**	**Totals**
Men	0	126	543	789	694	293	38	2,483
Women	6	113	692	835	740	242	13	2,641
Totals	6	239	1,235	1,624	1,434	535	51	5,124

Framingham Risk Score – 10 yr probability of having a complication from coronary heart disease



1 CHD Risk Factors

Age		
Years	LDL Pts	Chol Pts
30-34	-1	[-1]
35-39	0	[0]
40-44	1	[1]
45-49	2	[2]
50-54	3	[3]
55-59	4	[4]
60-64	5	[5]
65-69	6	[6]
70-74	7	[7]

2 LDL-C

(mg/dL)	(mmol/L)	LDL Pts
<100	<2.59	-3
100-129	2.60-3.36	0
130-159	3.37-4.14	0
160-190	4.15-4.92	1
≥190	≥4.92	2

Cholesterol

(mg/dL)	(mmol/L)	Chol Pts
<160	<4.14	[-3]
160-199	4.15-5.17	[0]
200-239	5.18-6.21	[1]
240-279	6.22-7.24	[2]
≥280	≥7.25	[3]

3 HDL-C

(mg/dL)	(mmol/L)	LDL Pts	Chol Pts
<35	<0.90	2	[2]
35-44	0.91-1.16	1	[1]
45-49	1.17-1.29	0	[0]
50-59	1.30-1.55	0	[0]
≥60	≥1.56	-1	[-2]

4 Blood Pressure

Systolic (mm Hg)	<80	80-84	85-89	90-99	≥100
<120	0 [0] pts				
120-129		0 [0] pts			
130-139			1 [1] pts		
140-159				2 [2] pts	
≥160					3 [3] pts

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

5 Diabetes

	LDL Pts	Chol Pts
No	0	[0]
Yes	2	[2]

6 Smoker

	LDL Pts	Chol Pts
No	0	[0]
Yes	2	[2]

7 Adding Up the Points

Age	_____
LDL-C or Chol	_____
HDL-C	_____
Blood Pressure	_____
Diabetes	_____
Smoker	_____
Point Total	_____

Relative Risk

- Very Low
- Low
- Moderate
- High
- Very High

8 Absolute CHD Risk

LDL Pts Total	10 Yr CHD Risk	Chol Pts Pts	10 Yr CHD Risk
<-3	1%		
-2	2%		
-1	2%	[<-1]	[2%]
0	3%	[0]	[3%]
1	4%	[1]	[3%]
2	4%	[2]	[4%]
3	6%	[3]	[5%]
4	7%	[4]	[7%]
5	9%	[5]	[8%]
6	11%	[6]	[10%]
7	14%	[7]	[13%]
8	18%	[8]	[16%]
9	22%	[9]	[20%]
10	27%	[10]	[25%]
11	33%	[11]	[31%]
12	40%	[12]	[37%]
13	47%	[13]	[45%]
≥14	≥56%	[≥14]	[≥53%]

9 Comparative Risk

Age (Years)	Average 10 Yr CHD Risk	Average 10 Yr Hard CHD Risk	Low 10 Yr CHD Risk
30-34	3%	1%	2%
35-39	5%	4%	3%
40-44	7%	4%	4%
45-49	11%	8%	4%
50-54	14%	10%	6%
55-59	16%	13%	7%
60-64	21%	20%	9%
65-69	25%	22%	11%
70-74	30%	25%	14%

Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837-47

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults

- Reviewed epidemiology and trials related to cholesterol and CHD
- 10 year CHD risk based on Framingham Risk Score
  - Online calculator 10 year risk NIH
  - <10% → goal LDL < 160 (drugs 190)
  - 10-20% → goal LDL < 130 (drugs 160)
  - >20% or diabetes → goal LDL < 100 (drugs 130)
- Majority in risk stratum < 10%

Predicting the 30-Year Risk of Cardiovascular Disease
The Framingham Heart Study

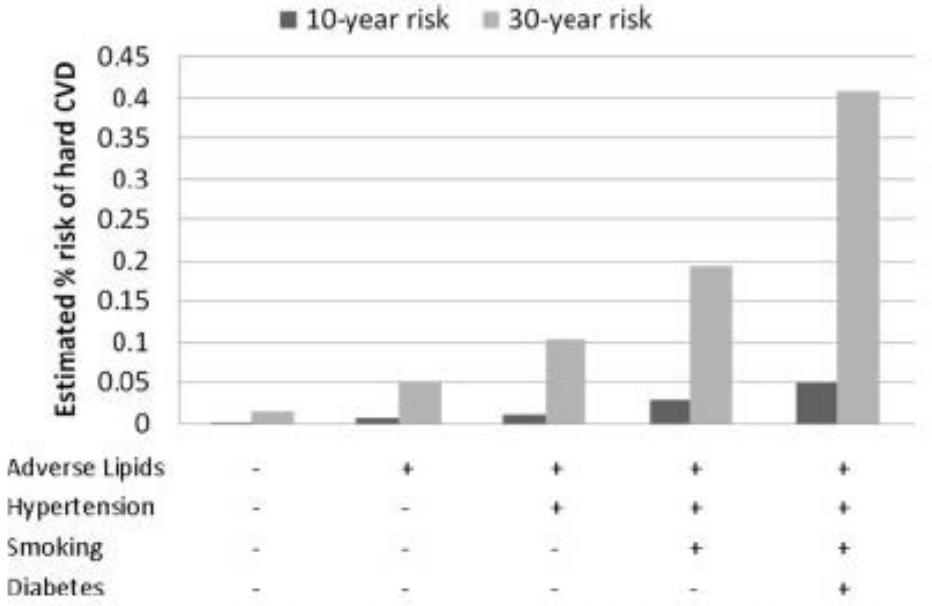


Figure 4. Ten- vs 30-year risk of hard CVD for 25-year-old men with different risk profiles. No risk factors profile: total cholesterol=150 mg/dL; HDL cholesterol=60 mg/dL; untreated SBP=110 mm Hg; nonsmoker; nondiabetic. Adverse lipids: total cholesterol=260 mg/dL; HDL cholesterol=35 mg/dL. Hypertension: SBP=160 mm Hg, untreated.

Pencina MJ, D'Agostino RB, Sr., Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the framingham heart study. Circulation 2009;119:3078-84

Predicting the 30-Year Risk of Cardiovascular Disease
The Framingham Heart Study

Table 2. Hazard Ratios With 95% CIs for 30-Year Risk of Hard CVD

Variables	Main Model	Simple Model
Male sex	1.73 (1.45, 2.07)	2.08 (1.77, 2.46)
Age	2.09 (1.88, 2.31)	2.22 (2.01, 2.45)
SBP	1.29 (1.19, 1.39)	1.26 (1.16, 1.36)
Antihypertensive treatment	1.48 (1.10, 2.00)	1.48 (1.09, 2.00)
Smoking	2.01 (1.72, 2.35)	2.21 (1.90, 2.58)
Diabetes mellitus	2.49 (1.82, 3.41)	2.82 (2.07, 3.84)
Total cholesterol	1.33 (1.23, 1.44)	. . .
HDL cholesterol	0.78 (0.72, 0.84)	. . .
BMI	. . .	1.20 (1.10, 1.30)

Hazard ratios for continuous risk factors are given per 1-SD increase in the natural logarithm. All $P \leq 0.01$.

Link for excel spreadsheet calculators of 30 year risk (main and simple models)

Pencina MJ, D'Agostino RB, Sr., Larson MG, Massaro JM, Vasan RS. Predicting the 30-year risk of cardiovascular disease: the framingham heart study. Circulation 2009;119:3078-84

Outline

- Epidemiology of Coronary artery disease
- Pathophysiology of coronary atherosclerosis
  - "CAD", hardening of arteries
- Heritability of CHD and risk factors
- GWAS discoveries
- Current risk prediction for CHD
  - Clinical considerations
- Old and new metrics for assessing improvement in risk prediction algorithms
  - Bringing in genetics
- Future Directions

How do we best assess the added usefulness of new biomarkers?

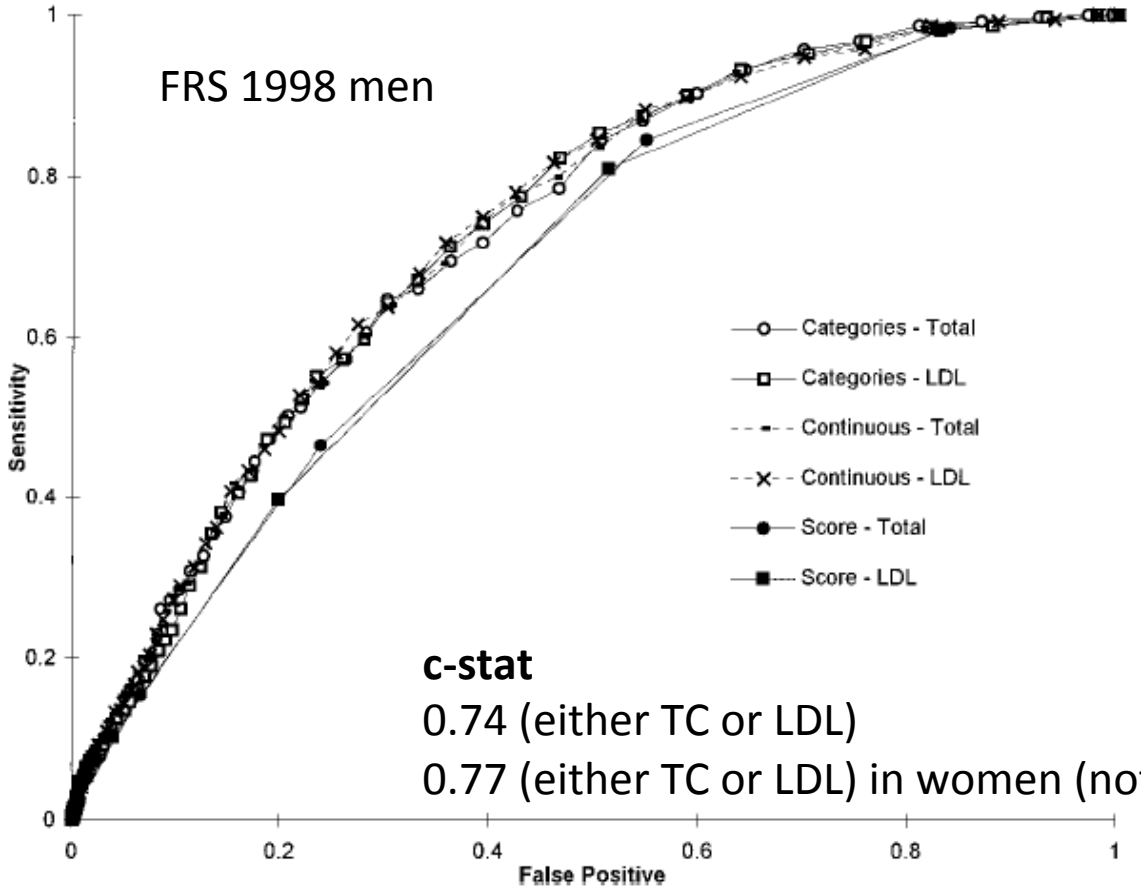
- Necessary prerequisite
  - Statistically significant association with outcome with descent effect size
- But not sufficient
  - Must show that your new model
    - remains well calibrated (predicted risks match observed)
    - Has an improved ability to discriminate cases & non-cases including appropriate reclassification among clinically meaningful categories of risk if they exist

How will we prove that bringing in genetic data to these models makes a difference?

- model assessment with and without genetic data (or any new markers)
  - 2 classes of metrics
    - DISCRIMINATION: most popular metric
      - Area under the Receiver Operating Characteristics (AUC) and c-statistic (borrowed from diagnostic testing)
      - Based solely on ranking cases and non cases and does not take absolute risks into consideration (which clinically are more important)
    - CALIBRATION: how well your predicted risk matches the observed risk for individuals

Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928-35.

AUC tough to budge when its already reasonably good



c-stat

0.74 (either TC or LDL)

0.77 (either TC or LDL) in women (not shown)

Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837-47

AUC tough to budge when its already reasonably good

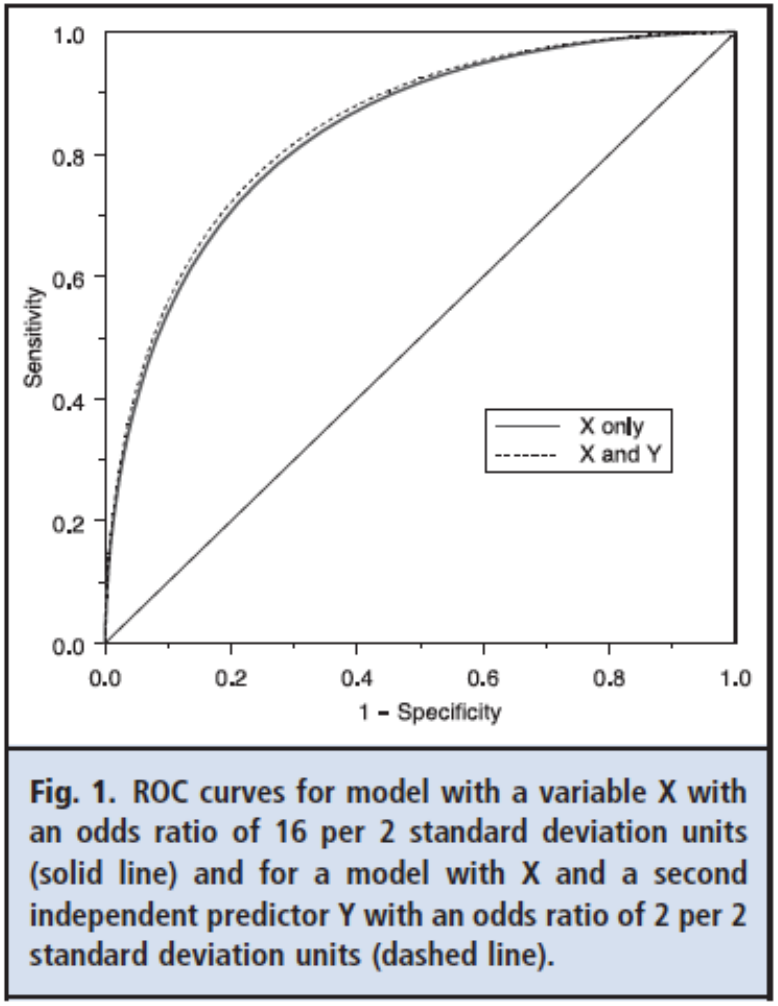


Fig. 1. ROC curves for model with a variable X with an odds ratio of 16 per 2 standard deviation units (solid line) and for a model with X and a second independent predictor Y with an odds ratio of 2 per 2 standard deviation units (dashed line).

Cook NR. Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. Clin Chem 2008;54:17-23.

Pitfalls of AUC / c-statistic metric

TABLE 1. Contributions to Cardiovascular Disease Prediction in the Women's Health Study*

Variable	Variable χ^2	*P*	RR per 2 SD†	*c* Statistic
Effect of adding variables to model with age only				
Ln(age) only	395.9	<0.0001	4.0	0.70
+Ln(SBP)	148.8	<0.0001	2.5	0.74
+Current smoking†	121.1	<0.0001	2.9	0.73
+Ln(HDL)	85.7	<0.0001	1/2.0	0.73
+Ln(TC)	33.3	<0.0001	1.6	0.72
+LDL	28.8	<0.0001	1.5	0.71
Effect of adding variables to model with age, SBP, and smoking				
Ln(age), Ln(SBP), smoking	...	...	...	0.76
+Ln(HDL)	45.3	<0.0001	1/1.7	0.77
+Ln(TC)	21.5	<0.0001	1.4	0.77
+LDL	18.6	<0.0001	1.4	0.77
Effect of deleting variables from full model				
Ln(age), Ln(SBP), smoking, Ln(TC), Ln(HDL)−	...	...	...	0.78
−Ln(TC)	39.8	<0.0001	1.6	0.77
−Ln(HDL)	63.6	<0.0001	1/1.7	0.77
−Current smoking	99.7	<0.0001	2.6	0.76
−Ln(SBP)	114.1	<0.0001	2.2	0.76
−Ln(Age)	257.4	<0.0001	3.2	0.73

+ indicates the addition of each variable separately to the model with age only, or age, SBP, and smoking only; −, the deletion of each variable separately from the full model; Ln, natural logarithms; RR, relative risk; and TC, total cholesterol. χ^2 is the likelihood ratio statistic for each single variable when added to the model (top and middle) or subtracted from the full model (bottom). Ln were used to normalize distributions and improve the fit for individual predictors.

*Estimated from Cox proportional hazards models.

†RR is relative risk when each variable is included in models shown in the top and middle sections, and is relative risk in full model in the bottom section; RR compares risk across 2 SD units, except for smoking, which is yes versus no.

Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928-35.

Assessment of risk prediction

- For many years → AUC
  - Insensitive to changes in absolute risk
  - Proven to be very hard to improve esp. in CAD
  - Even modifiable TRF minimally change AUC
- Newer metrics
    - Reclassification calibration (Cook)
    - Integrated discrimination improvement (IDI) (Pencina et al.)
    - Discrimination improvement (Pencina et al.)
      - Net reclassification index (nRCI)

Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928-35.

Cook NR, Paynter NP. Performance of reclassification statistics in comparing risk prediction models. Biom J 2011

New discrimination tests…

- Newly described set of discrimination tests that consider absolute predicted risk of individuals ("reclassification")
  - Net reclassification index (NRI)
  - Integrated discrimination index (IDI)
  - Others
- US Preventative Services Task Force has endorsed reclassification as important metric for prognostic tests

Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157-72; discussion 207-12.
Helfand M, Buckley DI, Freeman M, et al. Emerging risk factors for coronary heart disease: a summary of systematic reviews conducted for the U.S. Preventive Services Task Force. Ann Intern Med 2009;151:496-507.

Net Reclassification Index

- Must have a priori defined categories of clinical risk that are clinical meaningful and actionable
  - E.g. ATP III 0-10%, 11-20%, >20%
- Stratify your cohort by cases and non cases at end of follow up

Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157-72; discussion 207-12.

General concept of reclassification
Who moves and to where with new model?

TABLE 3. Comparison of Observed and Predicted Risks Among Women in the Women's Health Study*

	Model With HDL 10-Year Risk (%)				
Model Without HDL 10-Year Risk (%)	0 to <5%	5 to <10%	10 to <20%	20%+	% Reclassified
0% to <5%					
Total, n	22655	696	6	0	...
%†	97.0	3.0	0.0	0.0	3.0
Observed 10-year risk (%)‡	1.5	5.9	0.0	...	...
5% to <10%					
Total, n	593	1712	291	0	...
%	22.8	66.0	11.2	0.0	34.0
Observed 10-year risk (%)	3.7	7.6	14.7	...	...
10% to <20%					
Total, n	3	214	512	76	...
%	0.4	26.6	63.6	9.4	36.4
Observed 10-year risk (%)	0.0	7.5	10.7	23.3	...
20%+					
Total, n	0	0	41	102	
%	0.0	0.0	28.7	71.3	28.7
Observed 10-year risk (%)	...	...	15.8	32.5	...

*This comparison uses models that include Framingham risk factors with and without HDL. All estimated and observed risks represent 10-year risk of cardiovascular disease.

†Percent classified in each risk stratum by the model with HDL.

‡Observed proportion of participants developing cardiovascular disease in each category.

But what if you moved a subject that ended up a case into a lower category?

Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928-35.

Net Reclassification Index

1. {# of cases whose predicted risk with new model reclassifies them into a ↑ category of risk (appropriate) – # of cases whose predicted risk with new model reclassifies them into ↓ category (inappropriate)} ÷ BY TOTAL # OF CASES.

2. {# of non-cases whose predicted risk with new model reclassifies them into a ↓ category of risk (appropriate) – # of non-cases whose predicted risk with new model reclassifies them into a ↑ category (inappropriate)} ÷ BY TOTAL # OF CONTROLS.

3. 1. + 2. to calculate the "net" reclassification
   - If net +ve then good (ideally positive for both 1 and 2)

Pencina MJ, D'Agostino RB, Sr., D'Agostino RB, Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157-72; discussion 207-12.

A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses

	Region	Candidate gene(s)	Weight†	Reference†	Risk allele	Risk allele frequency	Other allele	Coronary heart disease (total n=19790)	
								Pooled HR (95% CI)‡	p value
rs17465637	1q41	*MIA3*	1·14	15	C	0·75	A	0·99 (0·87–1·12)	0·854
rs11206510	1p32	*PCSK9*	1·15	15	T	0·84	C	0·94 (0·81–1·09)	0·431
rs646776	1p13	*CELSR2–PSRC1–SORT1*	1·19	15	T	0·79	C	0·96 (0·84–1·09)	0·512
rs6725887	2q33	*WDR12*	1·17	15	C	0·11	T	1·14 (0·96–1·35)	0·126
rs9818870	3q22	*MRAS*	1·15	16	T	0·10	C	0·88 (0·73–1·06)	0·174
rs3798220	6q26	*LPA*	1·68	18	C	0·01	T	2·07 (1·39–3·09)	$3·8\times10^{-4}$
rs9349379	6p24	*PHACTR1*	1·12	15	C	0·44	T	1·16 (1·04–1·29)	0·008
rs4977574	9p21	*CDKN2A–CDKN2B*	1·29	15	G	0·43	A	1·21 (1·08–1·34)	0·001
rs1746048	10q11	*CXCL12*	1·17	15	C	0·84	T	1·13 (0·97–1·33)	0·113
rs2259816	12q24	*HNF1A*	1·08	16	T	0·36	G	1·02 (0·91–1·14)	0·774
rs3184504	12q24	*SH2B3*	1·13	17	T	0·40	C	1·03 (0·92–1·15)	0·568
rs1122608	19p13	*LDLR*	1·15	15	G	0·79	T	1·00 (0·87–1·14)	0·988
rs9982601	21q22	*SLC5A3–MRPS6–KCNE2*	1·20	15	T	0·14	C	1·29 (1·07–1·57)	0·009

SNP=single nucleotide polymorphism. HR=hazard ratio. *Association tested with Cox proportional hazards model adjusted for sex, LDL and
blood pressure, blood pressure treatment, and diabetes; age was used as the timescale. †SNP specific weights for genetic risk score calculatio
‡Results from FINRISK 1992, 1997, and 2002, Health 2000, and Malmö Diet and Cancer Cardiovascular Cohort were combined with fixed effe

Table 2: Association between SNPs and incident coronary heart disease, cardiovascular disease, and myocardial infarction*

Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;376:1393-400.

A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses

	Genetic risk score quintile					p value for trend
	1 (reference)	2	3	4	5	
HR (95% CI) for CHD (total n=25 243)						
FR 1992	1·00	0·97 (0·65–1·45)	1·07 (0·71–1·60)	1·60 (1·10–2·34)	1·54 (1·06–2·25)	0·001
FR 1997	1·00	1·02 (0·72–1·44)	1·17 (0·84–1·62)	1·32 (0·95–1·83)	1·76 (1·28–2·41)	$1·1\times10^{-4}$
FR 2002	1·00	1·06 (0·56–1·99)	1·18 (0·65–2·15)	1·43 (0·79–2·58)	1·82 (1·03–3·22)	0·019
Health 2000	1·00	0·93 (0·51–1·68)	1·41 (0·81–2·45)	1·13 (0·62–2·06)	1·51 (0·87–2·62)	0·087
Pooled†	1·00	1·00 (0·80–1·25)	1·17 (0·94–1·46)	1·39 (1·12–1·72)	1·66 (1·35–2·04)	$7·3\times10^{-6}$

Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;376:1393-400.

A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses

	Genetic risk score 0–5%	Genetic risk score 5–10%	Genetic risk score 10–20%	Genetic risk score >20%
Cases, by predicted risk*				
0–5%	82 (93%)	6 (7%)	0	0
5–10%	10 (8%)	100 (84%)	9 (8%)	0
10–20%	0	9 (5%)	136 (81%)	22 (13%)
>20%	0	0	9 (7%)	129 (93%)
Non-cases, by predicted risk*				
0–5%	9224 (99%)	121 (1%)	0	0
5–10%	179 (12%)	1149 (79%)	131 (9%)	0
10–20%	0	120 (14%)	687 (80%)	49 (6%)
>20%	0	0	54 (13%)	351 (87%)

*10-year predicted risk on the basis of traditional risk factors only.

Total cases: 512

Total non cases: 12065

	Individuals reclassified in table 5		NRI		
	Up	Down	Value	95% CI	p value
Cases	37	28	0·018	−0·014 to 0·051	0·278
Non-cases	301	353	0·004	0·0001 to 0·008	0·038
Total	..	..	0·022	−0·010 to 0·055	0·182

NRI=net reclassification improvement. *Comparison of models with and without genetic risk score, adjusted for sex, LDL and HDL cholesterol, smoking, body-mass index, systolic and diastolic blood pressure, blood pressure treatment, and diabetes; age was used as the timescale. †NRI for the subset of participants in 5–20% risk category in the model without genetic risk score, with risk classes 0–5%, 5–20%, and >20%.[23]

Table 6: **Net reclassification improvement of genetic risk score for coronary heart disease in the FINRISK 1992 and 1997 cohorts***

Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;376:1393-400.

A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses

- Summary
  - No effect on AUC (0.871 to 0.872, p =0.19)
  - calibration of the models with (p=0·52) and without (p=0·47) genetic risk score was good
  - IDI 0·004, p=0·0006
  - No effect on NRI (2·2%, p=0·182)

Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;376:1393-400.

Conclusions similar in Framingham Heart Study

Table 2. The HRs for the Association Between a GRS (per Allele) and Incident Events

GRS	No. of SNPs	Age- and Sex-Adjusted HR (95% CI)	*P* Value	Risk Factor–Adjusted HR (95% CI)*	*P* Value	Fully Adjusted HR (95% CI)†	*P* Value
Incident hard CHD							
1	13	1.09 (1.02–1.17)	0.02	1.07 (1.00–1.15)	0.04	1.07 (1.00–1.15)	0.04
2	102	1.02 (0.99–1.04)	0.14	1.01 (0.99–1.03)	0.48	1.01 (0.99–1.03)	0.47
Incident CVD							
1	13	1.06 (1.02–1.10)	0.006	1.05 (1.01–1.09)	0.03	1.05 (1.01–1.09)	0.03
2	102	1.01 (1.00–1.03)	0.07	1.01 (1.00–1.02)	0.54	1.00 (0.99–1.02)	0.52

HR indicates hazard ratio; GRS, genetic risk score; SNP, single-nucleotide polymorphism; CHD, coronary heart disease; CVD, cardiovascular disease.

*Adjusted for age, sex, total cholesterol, high-density lipoprotein, presence of diabetes, systolic blood pressure (and antihypertensive treatment), and cigarette smoking.

†Adjusted for age, sex, total cholesterol, high-density lipoprotein, presence of diabetes, systolic blood pressure (and antihypertensive treatment), cigarette smoking, and parental history of CVD.

Thanassoulis, G., G. M. Peloso, et al. (2012). "A Genetic Risk Score is Associated with Incident Cardiovascular Disease and Coronary Artery Calcium - The Framingham Heart Study." Circ Cardiovasc Genet.

Conclusions similar in Framingham Heart Study

Table 4. Risk Reclassification for the Addition of the Coronary Disease GRS to a Model Predicting 10-Year Risk of Hard CHD

Model	NRI	95% CI	Event/Nonevent NRI	IDI	95% CI	ContNRI	95% CI
1							
Age and sex (reference model)	. . .	. . .	. . .	. . .	. . .	. . .	. . .
+GRS	0.043	(−0.003 to 0.088)	0.041/0.002	0.001	(0.001 to 0.002)	0.22	(0.057 to 0.377)
2							
Age, sex, and CVD risk factors (reference model)	. . .	. . .	. . .	. . .	. . .	. . .	. . .
+GRS	0.001	(−0.040 to 0.039)	0.0003/0.0005	0.001	(−0.001 to 0.003)	0.17	(0.010 to 0.328)
3							
Age, sex, CVD risk factors, and parental history (reference model)	. . .	. . .	. . .	. . .	. . .	. . .	. . .
+GRS	−0.01	(−0.052 to 0.033)	−0.011/0.001	0.001	(−0.001 to 0.003)	0.19	(0.024 to 0.344)

NRI is calculated for the addition of the GRS to a reference model with the following risk cutoffs for 10-year risk of hard CHD: low (<6%), intermediate (6%–20%), and high (>20%).

GRS indicates genetic risk score; CHD, coronary heart disease; NRI, net reclassification index; IDI, integrated discrimination index; contNRI, continuous cutoff independent form of the NRI; CVD, cardiovascular disease.[36]

"For risk reclassification, results for reclassification metrics using the 29-SNP GRS (including CARDIoGRAM loci) were similar to the results for the 13-SNP score data presented in Table 4."

Thanassoulis, G., G. M. Peloso, et al. (2012). "A Genetic Risk Score is Associated with Incident Cardiovascular Disease and Coronary Artery Calcium - The Framingham Heart Study." Circ Cardiovasc Genet.

Genetic Risk Scores to improve CAD prediction using validated SNPs: not ready for prime time yet

Why?

*Effect sizes too small

*Not enough of them

*Not enough genetic variance explained

What will it take?

*single variant with RR of >10

*~20% genetic variance explained

*~will genetic predictors of risk factors help even if risk factors themselves included in model

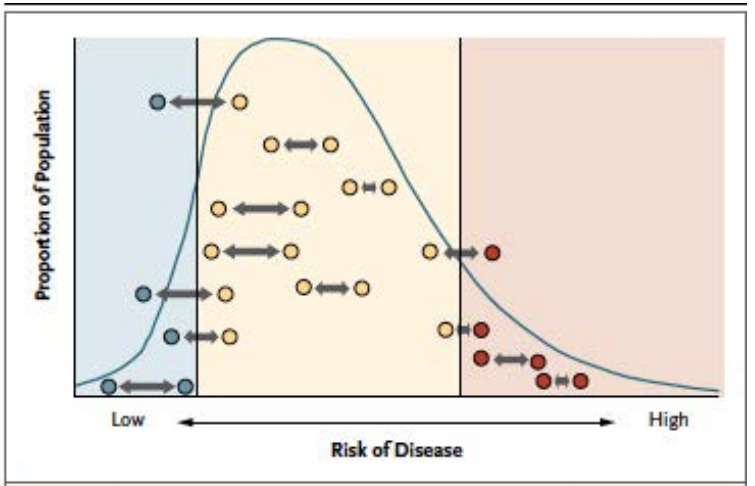


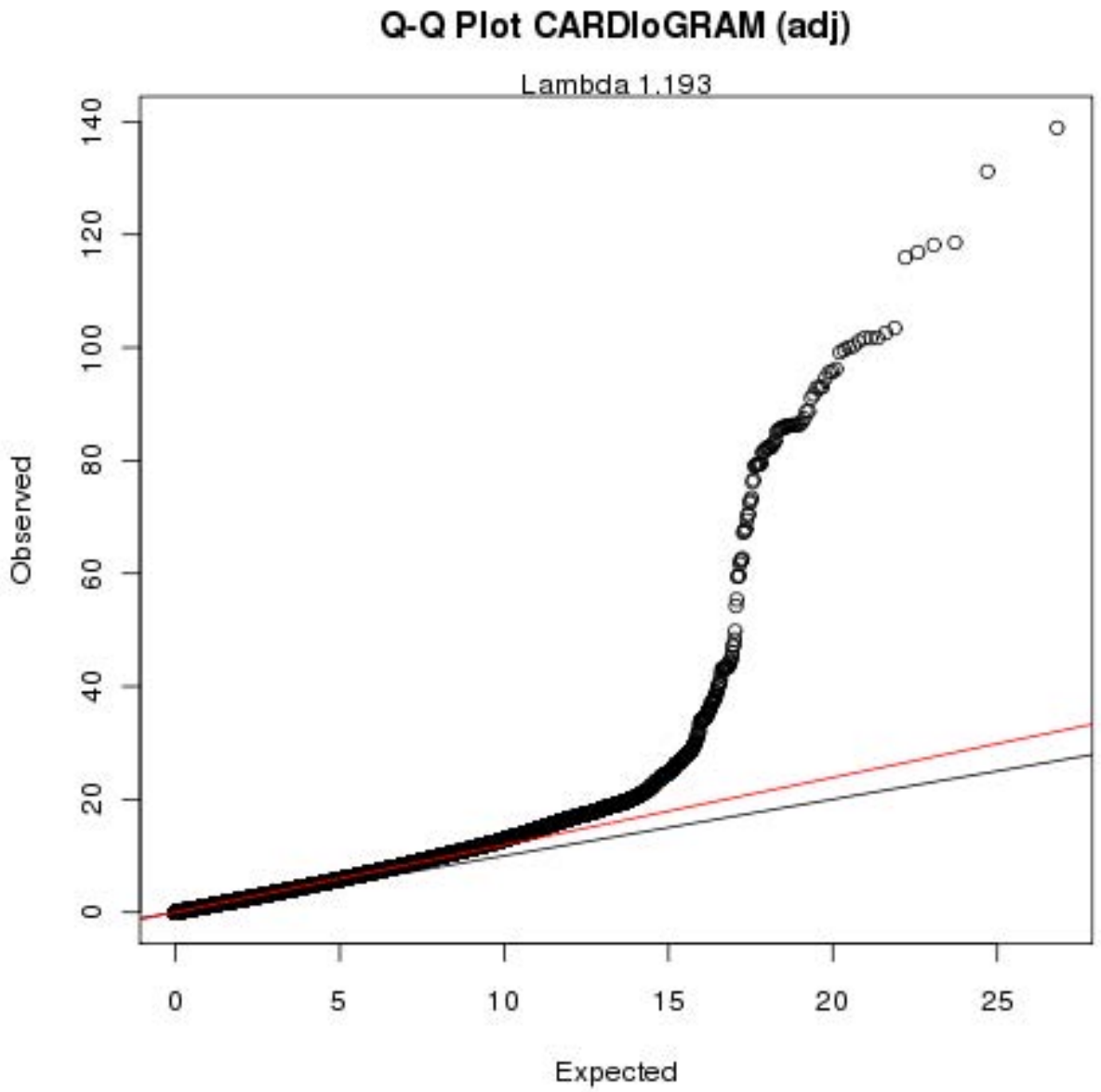
Figure 5. Reclassification of Persons at Various Levels of Risk, According to Risk Thresholds.

Thanassoulis G, Vasan RS. Genetic cardiovascular risk prediction: will we get there? Circulation 2010;122:2323-34.

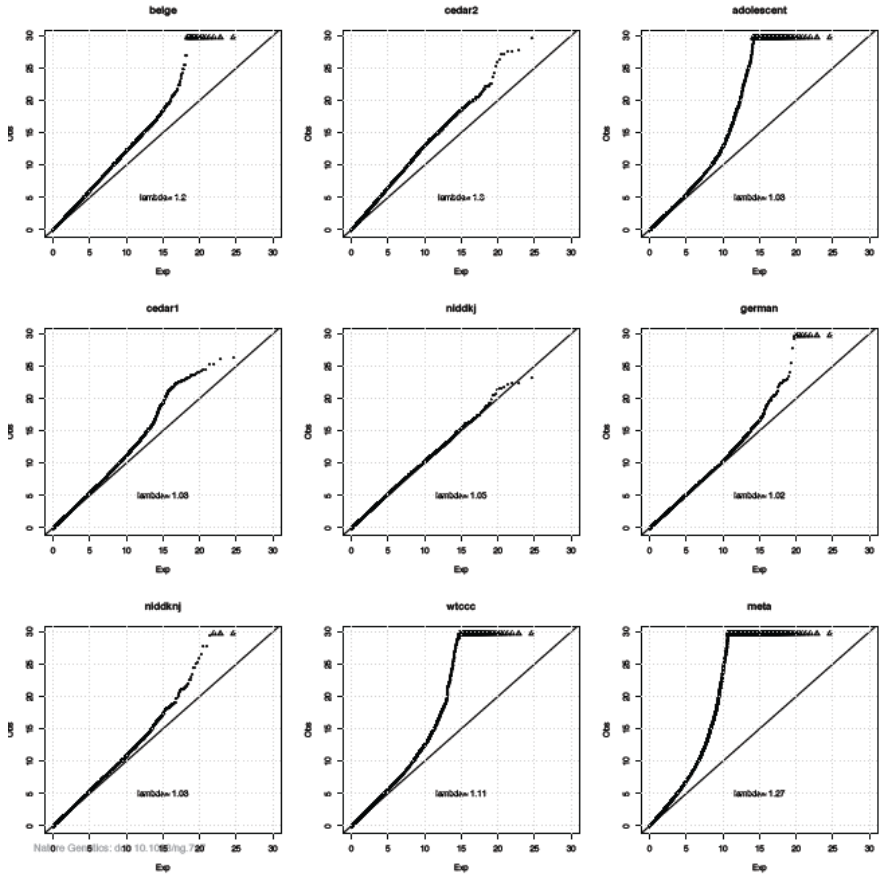
Manolio, T. A. (2010). "Genomewide association studies and assessment of the risk of disease." N Engl J Med **363(2): 166-176.**

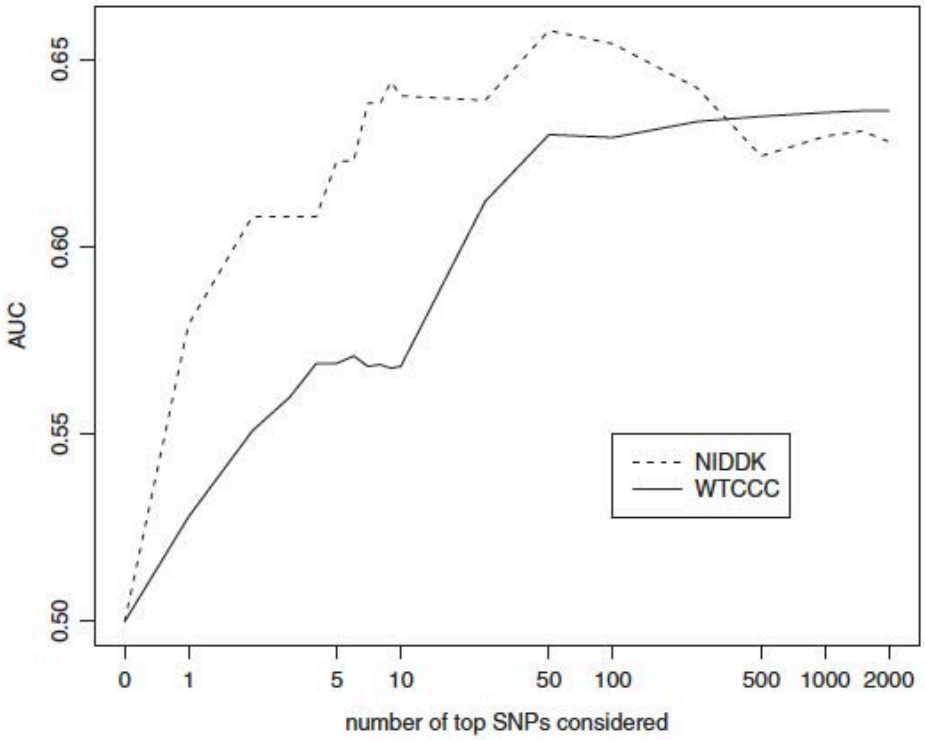
Future Directions

- Identify more of the "missing heritability"
  - More GWAS meta-analysis
    - Some have suggested tens to hundreds of loci with OR 1.05-1.10 remain
  - Metabochip (top hits risk factors of CAD and CARDIoGRAM)
  - Sequencing to look for rare variants
    - Known loci and novel loci
    - Will effect sizes be higher? How much higher?
  - Replication proving extremely challenging
- Testing multi locus genetic Risk Scores of top hits
- Coronary Artery Calcification
- Other race/ethnic groups
  - Fine mapping of known loci (metabochip)


Q-Q Plot CARDIoGRAM (adj)
Lambda 1.193
Observed
Expected
0
20
40
60
80
100
120
140
0
5
10
15
20
25

Improved risk prediction using top SNPs from GWAS even if not “validated”





Performance of multilocus GRS:

-more GWAS data is meta-analyzed?

-effect of addition of rarer variants?

Franke A, McGovern DP, Barrett JC, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nat Genet 2010;42:1118-25.

Kooperberg C, Leblanc M, Obenchain V. Risk prediction using genome-wide association studies. Genet Epidemiol 2010.

The very latest….CARDIoGRAM+C4D

Supplementary Table XX. Summary of model performance metrics for CARDIoGRAM+C4D derived Genetic Risk Scores in predicting incident coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study

	HR per SD of GRS (95% CI)	LR R^2	LR C-Stat	IDI* (CI)	IDI (P)	NRI* [0,.1,.2,1](CI)	NRI (P)
Age & Sex only model	--	0.077	0.674	--	--	--	--
+ GRS Table 1 SNPs	1.22 (1.15,1.30)	0.085	0.684	0.005 (0.003, 0.007)	1.30E-07	0.033 (0.005, 0.061)	0.020
+ GRS Table 1 & 2 SNPs	1.24 (1.17,1.32)	0.087	0.686	0.006 (0.004 (0.008)	2.29E-08	0.029 (-0.001, 0.059)	0.054
+ GRS All SNPs (FDR < 5%)	1.27 (1.19,1.35)	0.089	0.688	0.007 (0.005, 0.010)	6.21E-10	0.021 (-0.010, 0.052)	0.180
All traditional risk factors model	--	0.165	0.752	0.05 (0.05, 0.06)	1.91E-049	0.19 (0.14, 0.23)	7.12E-17
+ GRS Table 1 SNPs	1.21 (1.13,1.28)	0.171	0.757	0.005 (0.003, 0.007)	2.74E-006	0.001 (-0.022, 0.024)	0.925
+ GRS Table 1 & 2 SNPs	1.22 (1.15,1.30)	0.172	0.757	0.006 (0.003, 0.008)	1.14E-006	-0.002 (-0.025, 0.022)	0.889
+ GRS All SNPs (FDR < 5%)	1.24 (1.17,1.32)	0.174	0.758	0.007 (0.004, 0.009)	5.98E-008	0.005 (-0.021, 0.030)	0.718

GRS: Genetic Risc Score, HR: Hazard Ratio, 95% CI: 95% Confidence Intervals, OR: Odds Ratio, LR: logistic regression censoring at 10 years of follow up, C-stat: c statistic, IDI: integrated discrimination index, NRI: net reclassification index, when low, medium, and risk strata are defined as 0-10%, 11-20% ,>20%

Goldstein and Assimes, GRS analysis for main CARDIoGRAM+C4D paper

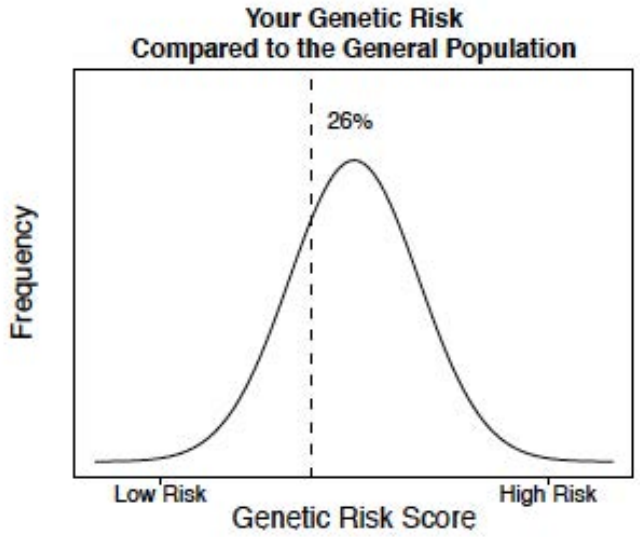
A randomized trial of personal genomics for preventive cardiology: design and challenges

Your Risk Score

Based on the traditional Framingham risk score, your risk of coronary heart disease over the next 10 years is approximately 9.7%.

We tested for a total of 38 possible risk variants or alleles. Out of these 38, you carry 15 variants that are associated with higher risk.

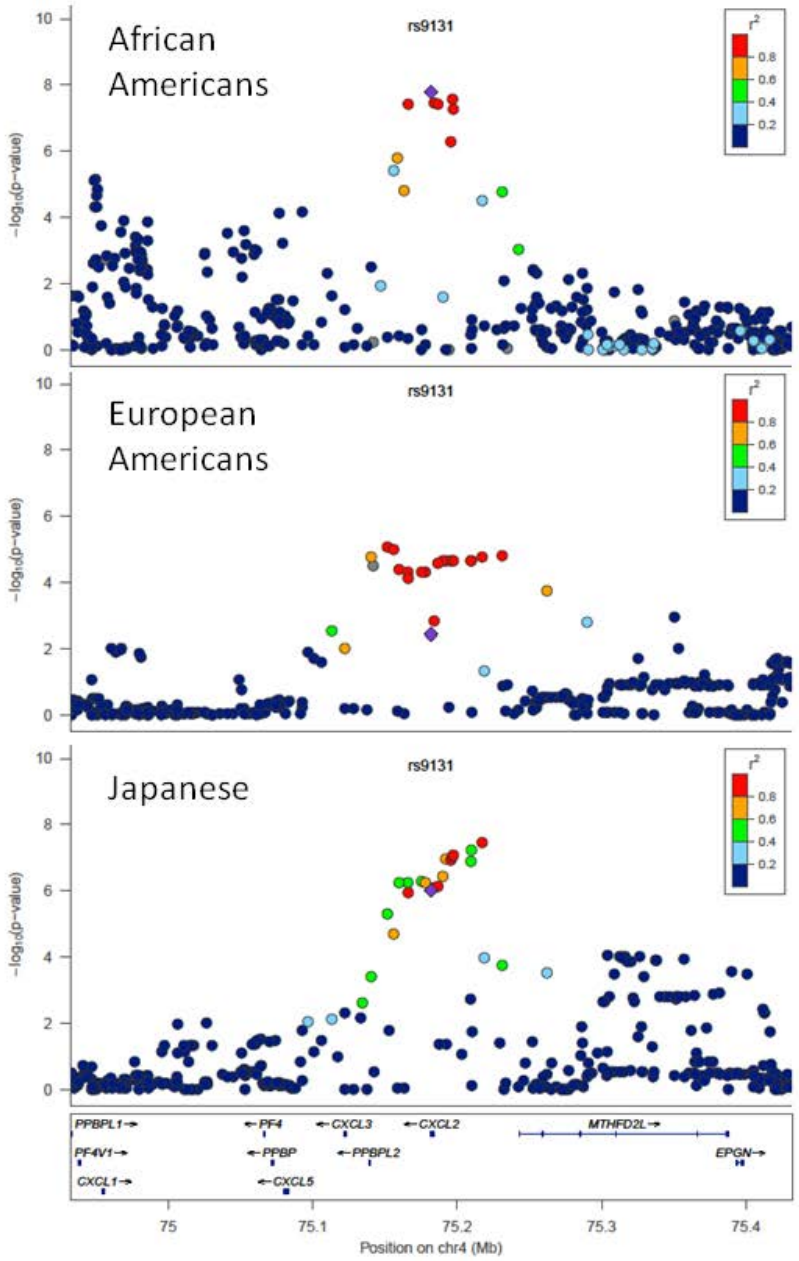
Your genetic profile puts you in the 26 percentile for risk. This means 26% of the general population have a genetic risk score more favorable than you and 74% have a genetic risk score less favorable than you.



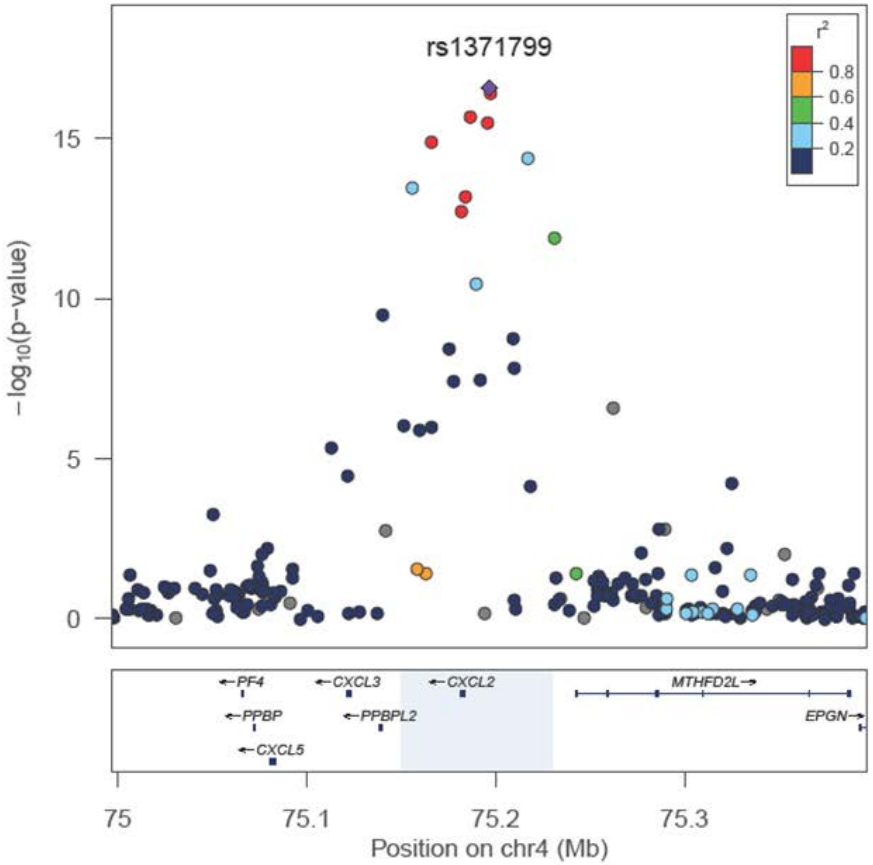
Based on the traditional Framingham risk score plus the genetic risk score, your risk of coronary heart disease over the next 10 years is approximately **8.7%**.

Knowles J, Assimes TL, et al. In press Circulation Cardiovascular Genetics

Fine Mapping through genotyping non European race-ethnic groups
Association of *CXCL2* rs9131 with WBC in 3 populations



Trans-population meta-analysis narrows *CXCL2* association signal



Reiner A. et al. Plos Genetics , under review

Table 4 Quintiles of allelic dosage score comprised of nine validated SNPs and risk for early-onset myocardial infarction

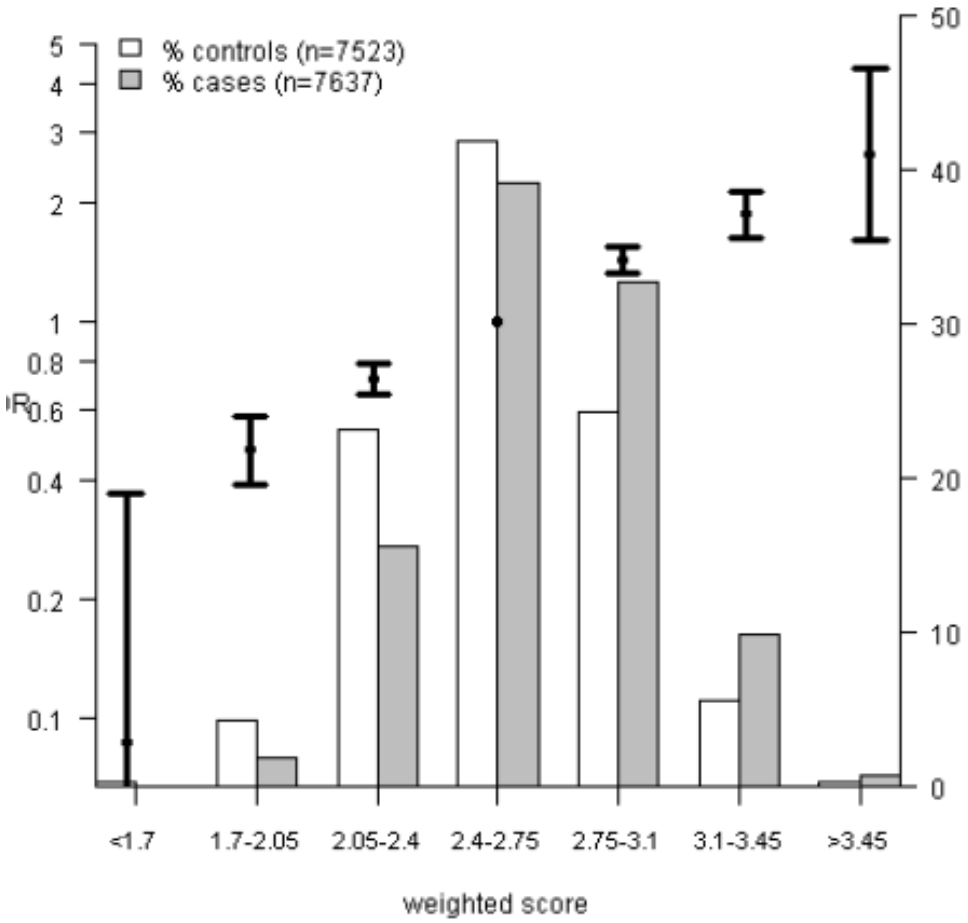
Quintile of myocardial infarction genotype score	Odds ratio	95% confidence interval
Quintile 1	1.0 (reference group)	
Quintile 2	1.22	1.04–1.44
Quintile 3	1.43	1.22–1.68
Quintile 4	1.69	1.44–1.99
Quintile 5	2.23	1.89–2.63
P for association of myocardial infarction genotype score with early-onset myocardial infarction: 2×10^{-28}		

The nine validated myocardial infarction polymorphisms are as shown in **Table 2** and **Table 3** and include *SLC5A3-MRPS6-KCNE2* rs9982601, *PHACTR1* rs12526453, *WDR12* rs6725887, 9p21.3 rs4977574, *CXCL12* rs1746048, *CELSR2-PSRC1-SORT1* rs646776, *MIA3* rs17465637, *LDLR* rs1122608, and *PCKS9* rs11206510. Risk of early-onset myocardial infarction was assessed in the 2,967 cases and 3,075 controls from stage 1.

Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;41:334-41.

CARDIoGRAM weighted risk score and risk of CAD

- range of # of high risk alleles: 15 to 37 (23 loci)
- mean weighted risk score cases higher than controls (P < 10−20)
- top 10%tile vs. 50%tile score → OR 1.88 (95% CI 1.67–2.11)
- bottom 10%ile vs.50%ile score → OR 0.55 (95% CI 0.48–0.64),
- model optimistic because discovery cohorts used to model



Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011

Reclassification Calibration

Table 1 Hypothetical example of reclassification data table with four risk strata

Model 1		Model 2				Total
		0 to <5%	5 to <10%	10 to <20%	20%+	
All						
0 to <5%	*N*	4564	416	36	2	5018
	Observed risk	0.020	0.053	0.222	–	
5 to <10%	*N*	738	977	375	32	2122
	Observed risk	0.034	0.075	0.123	0.125	
10 to <20%	*N*	76	451	763	288	1578
	Observed risk	0.053	0.086	0.166	0.243	
20%+	*N*	0	25	266	991	1282
	Observed risk	–	0.160	0.158	0.387	
Total		5378	1869	1440	1313	10000

Cook NR, Paynter NP. Performance of reclassification statistics in comparing risk prediction models. Biom J 2011

Net reclassification index

Table 1 Hypothetical example of reclassification data table with four risk strata

Model 1		Model 2				Total
		0 to <5%	5 to <10%	10 to <20%	20%+	
Cases						
0 to <5%	*N*	93	22	8	0	123
5 to <10%	*N*	25	73	46	4	148
10 to <20%	*N*	4	39	127	70	240
20%+	*N*	0	4	42	384	430
Total		122	138	223	458	941
Controls						
0 to <5%	*N*	4471	394	28	2	4895
5 to <10%	*N*	713	904	329	28	1974
10 to <20%	*N*	72	412	636	218	1338
20%+	*N*	0	21	224	607	852
Total		5256	1731	1217	855	9059

$$\text{NRI} = \frac{\text{Cases Up} - \text{Cases Down}}{\text{No. of cases in risk category}} - \frac{\text{Controls Up} - \text{Controls Down}}{\text{No. of controls in risk category}}$$