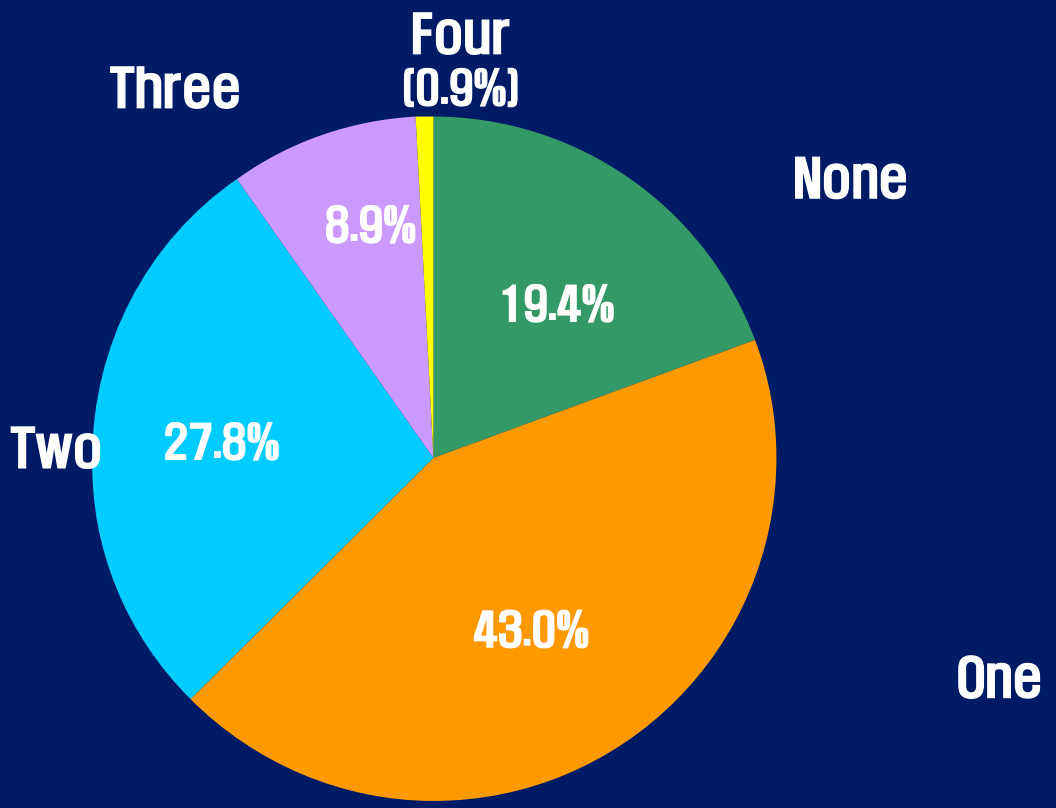
Gene and genomes in Coronary artery disease

Yangsoo Jang
Yonsei University College of Medicine
Cardiovascular Center
Division of Cardiology

허혈성 심장질환 환자들에서의 주요 위험요인 동반개수



Total patients=87 869
CHD=coronary heart disease
†smoking, hypertension, hypercholesterolaemia and diabetes mellitus

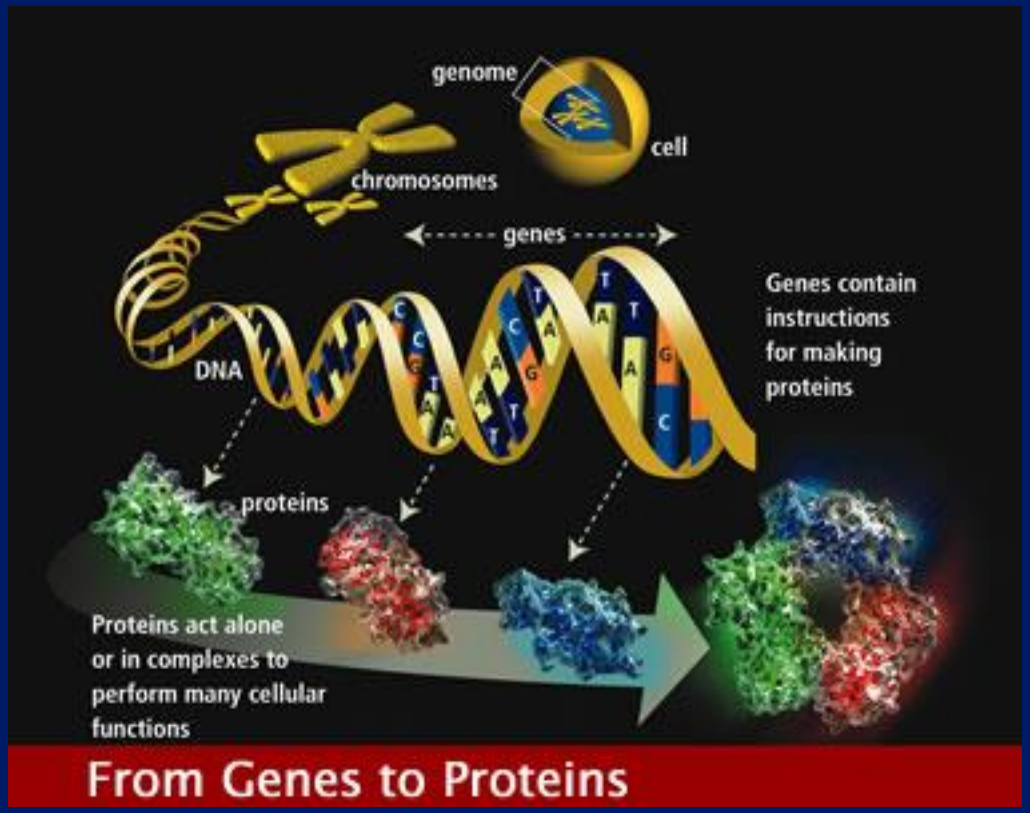
1/3 to 1/2 of CHD disease occurs in patients classified as low risk by conventional Risk factors

Khot UN *et al*. *JAMA* 2003; 290: 898–904

Genetic basis of CHD

- **Twin studies indicate that heritability of CVD is 30-60% (*N Engl J Med 1994;330:1041*)**
- **Heritability** as high as 63% for premature **MI** ***(Circulation 1980;61:503***)
- **History of premature death of a biological parent(<50 years) was associated with 4.5 fold increase in mortality for adopted offsprings → Risk not increased in adopted children with history of premature CAD in foster parents (*N Engl J Med 1988;318:727*)**

Human Genome Structure



chr #	Size (Mb)	Genes #
1	250	3511
2	243	2368
3	198	1926
4	191	1444
5	181	1633
6	171	2057
7	159	1882
8	146	1315
9	141	1534
10	136	1391
11	135	2168
12	134	1714
13	115	720
14	107	1532
15	103	1249
16	90	1326
17	81	1773
18	78	557
19	59	2066
20	63	891
21	48	450
22	51	855
X	155	1672
Y	59	429

Total genomic size = 3,106 Mb
Total number of genes = 36,464 genes

***Homo sapiens* Build 37.3 (Oct. 5, 2011)**

Human Genome Variations

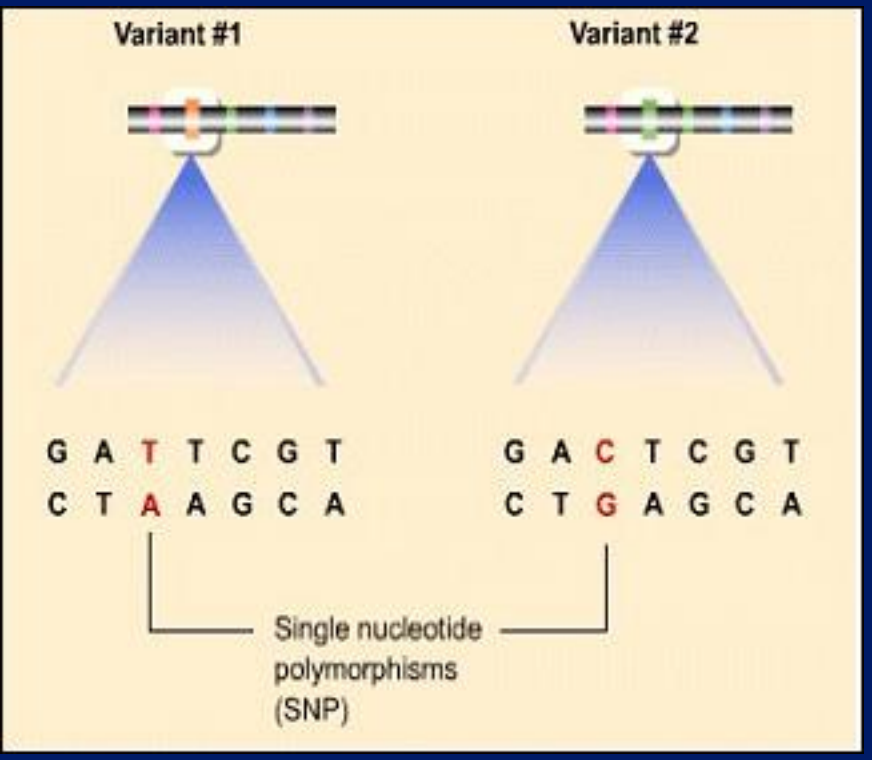
Genome Variation

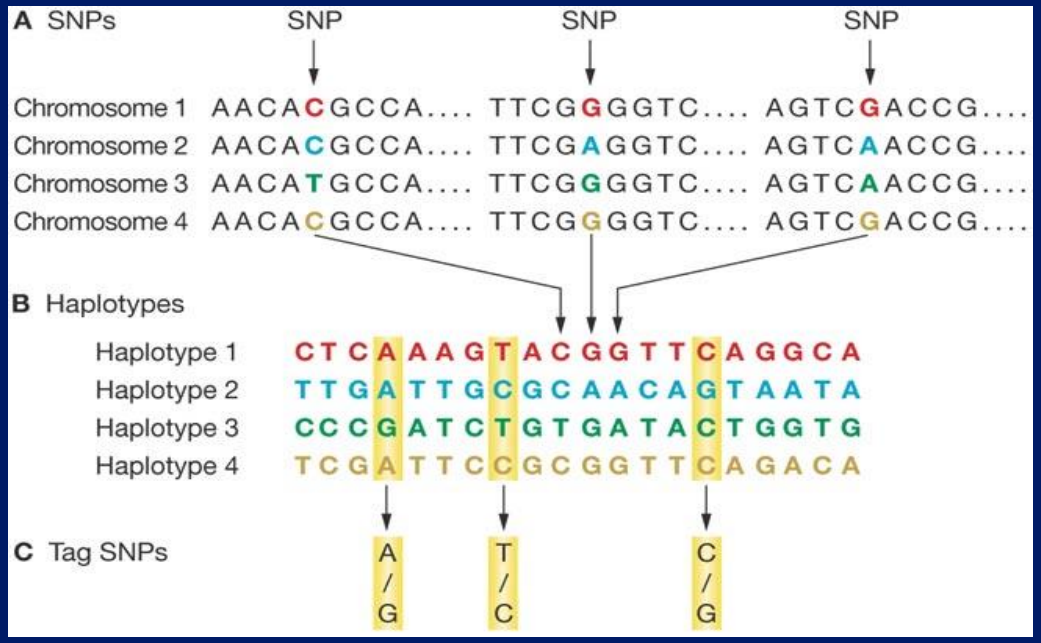
- Base Variation
  - Single Nucleotide Polymorphism (SNP)

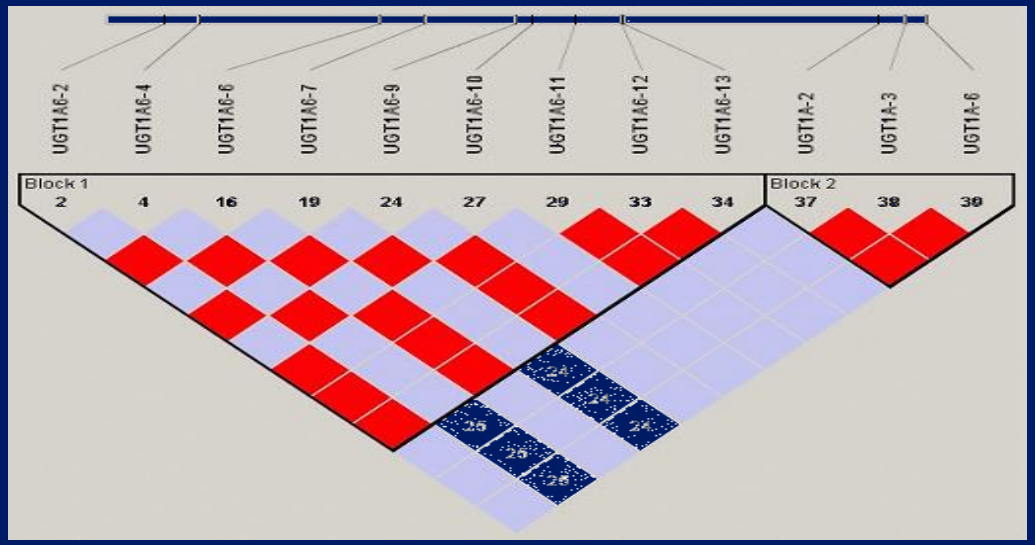
    ---ATGCGTG---
    ---ATGAGTG---
  - Insertion & Deletion(InDel)

    ---ATGCGTG---
    ---ATG—GTG---
- Structural Variation
  - Copy Number Variation (CNV)
    - Copy Number Gain
    - Copy Number Loss
  - Normal CNV, Structural Variation
    - Inversion
    - Translocation

SNP, Haplotype and TagSNPs

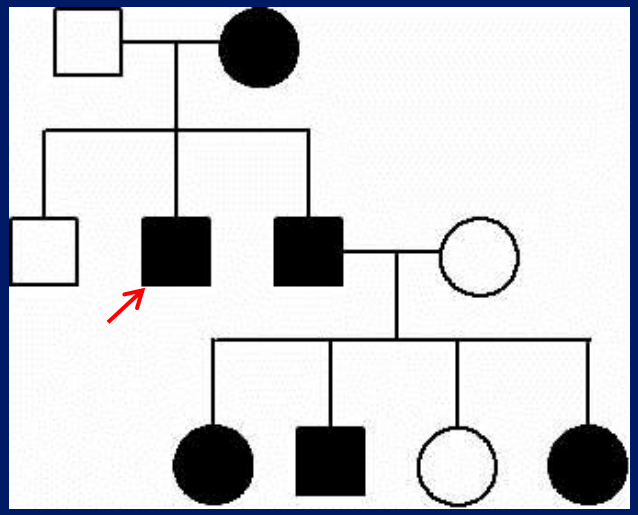






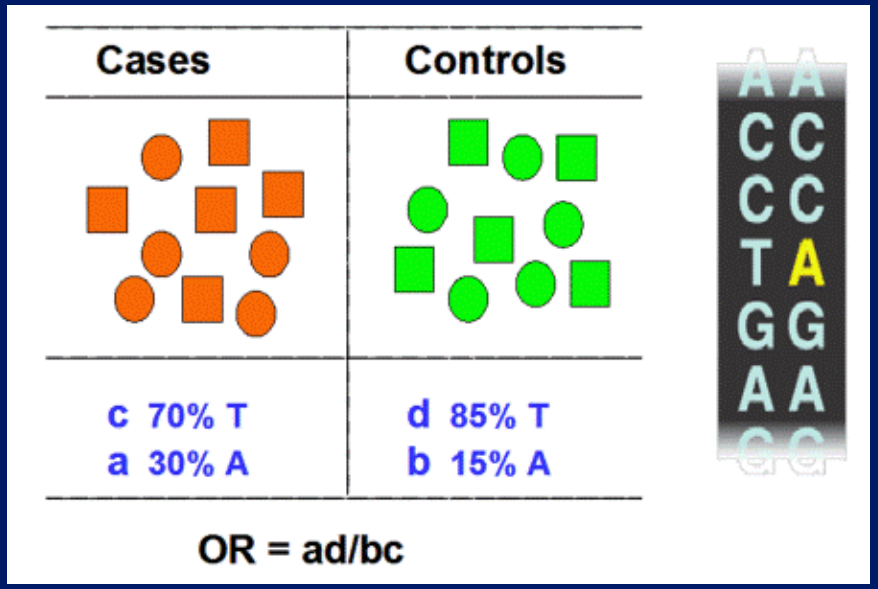
Strategies for Disease Gene Identification

Linkage Analysis (Family)



- Single gene
- Mendelian inheritance
- Rare, but high penetrance
- ~300-400 STR markers

Association Study (Population)



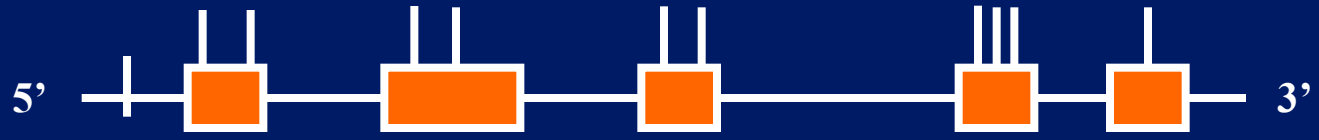
- Polygenic (also G X E)
- Complex inheritance
- Common
- Multiple polymorphic SNP markers

Approaches to determine susceptibility genes

- Candidate gene approach
- GWAS

Analytic Tools of Association Study

▪ Candidate genes approach



Catalog and test all coding SNPs for function

→ DNA Sequencing / SNapShot or TaqMan assay

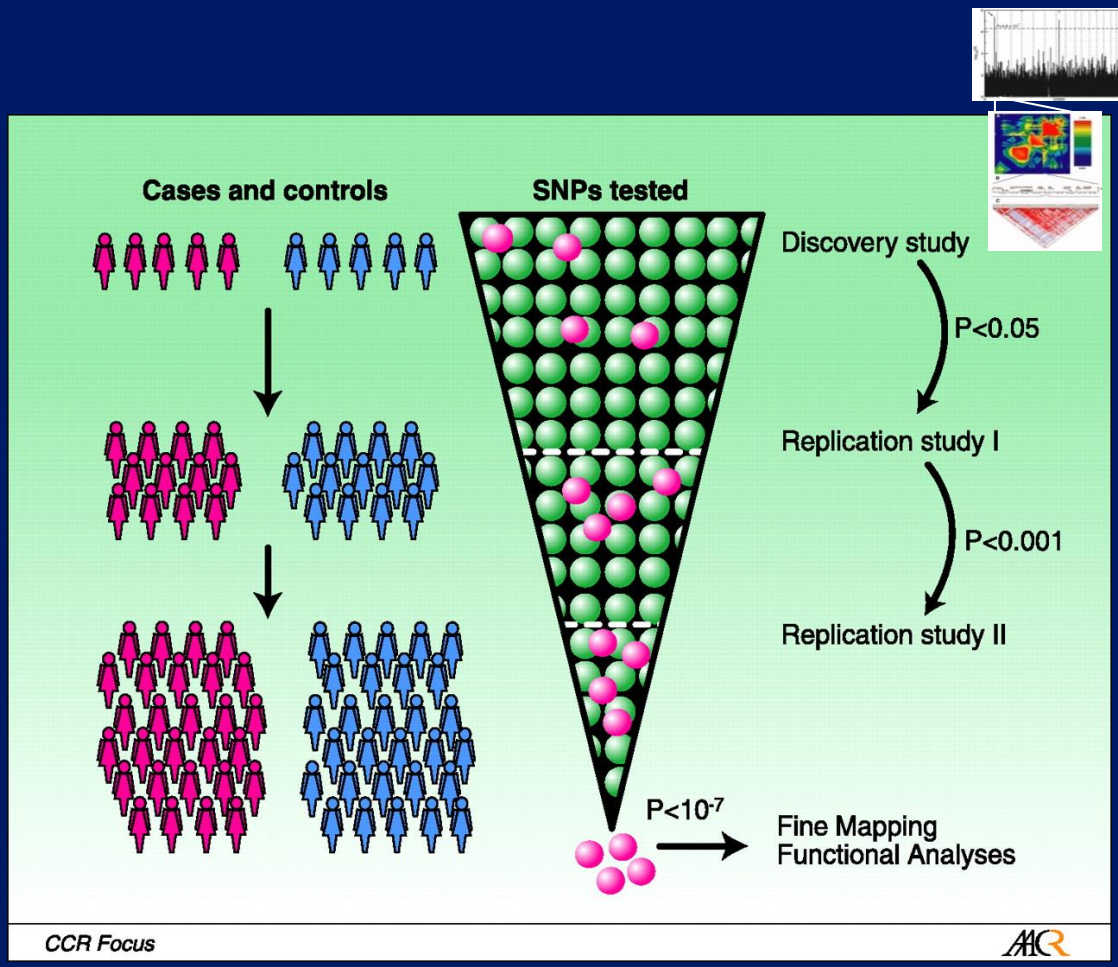
▪ Genome-Wide Association approach

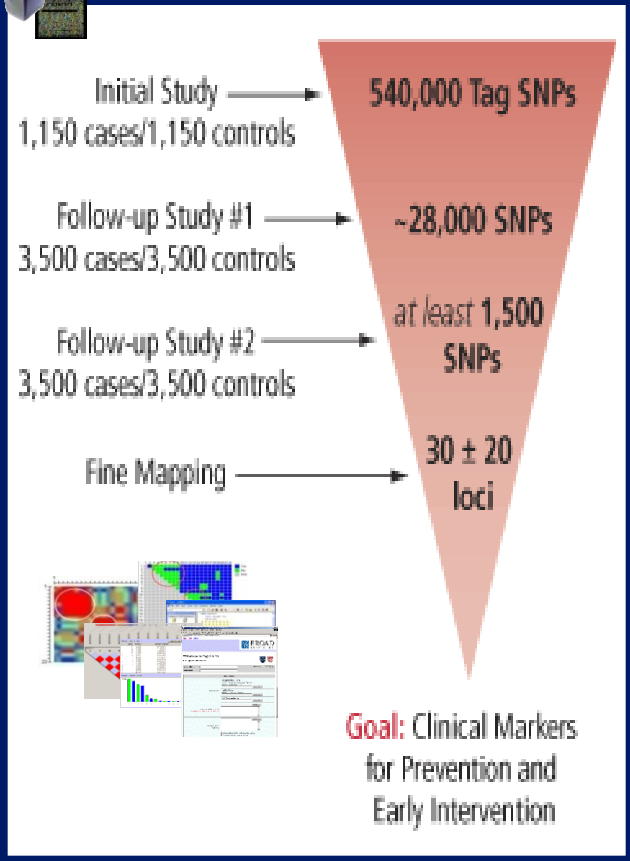
Use dense map of SNPs and test for LD (use association to find sites in entire sequence with function)

→ Affymetrix GeneChip / Illumina GeneChip

Genome-Wide Association Study (GWAS)

- **GWAS are used to identify common genetic factors (SNP, Ins/del) that influence health and disease.**

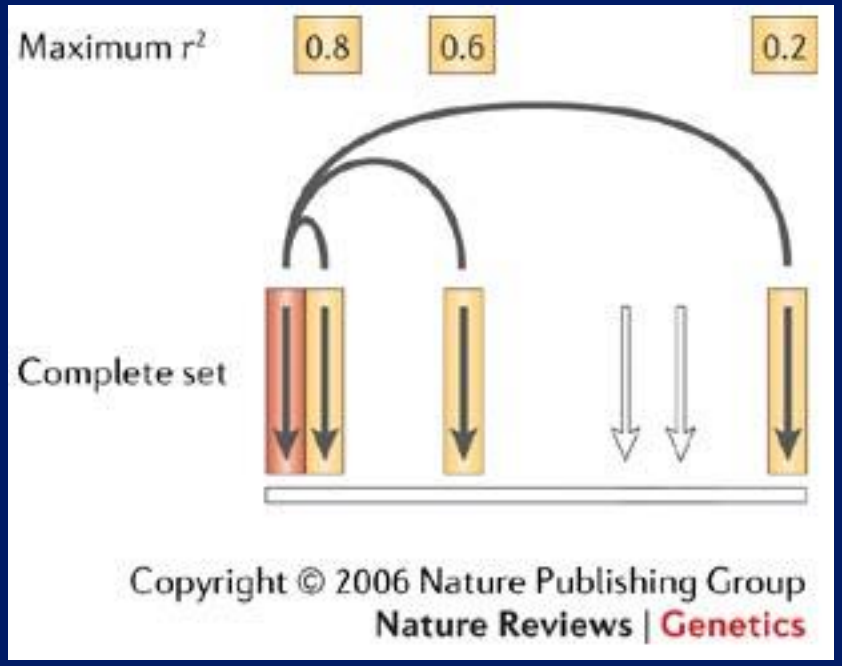




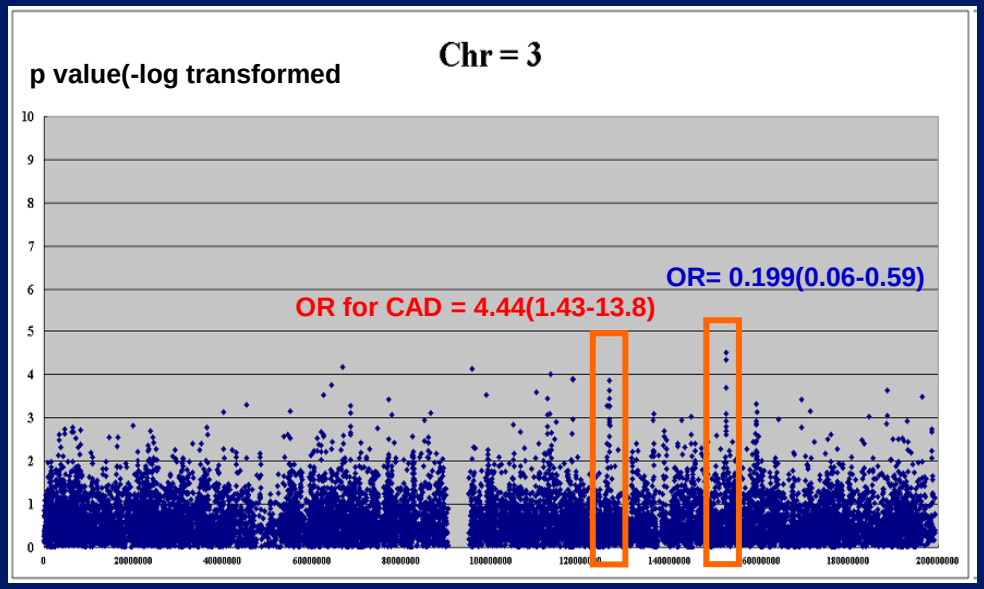
General GWAS strategy for common complex disease

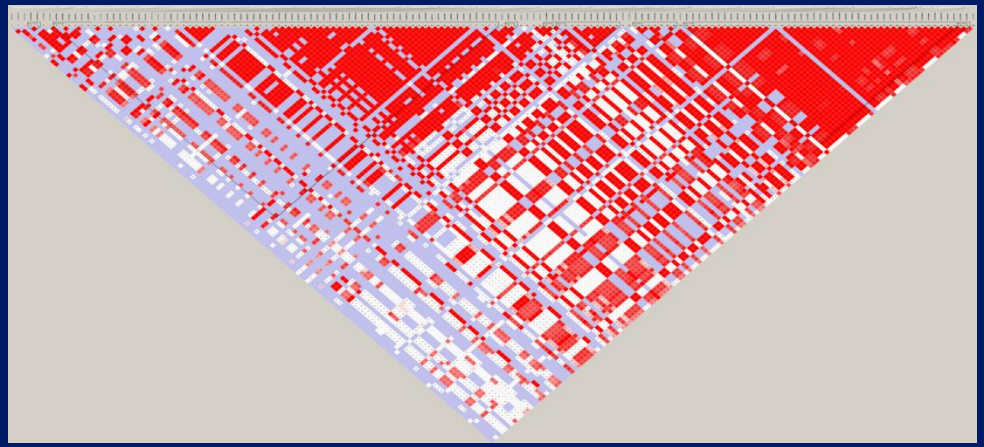
Linkage disequilibrium (LD) block & Association analysis

(A)



(B)





In 2007: Discovery of 9p21 and the watershed moment for cardiovascular genetics

Science
AAAS

A Common Variant on Chromosome 9p21 Affects the Risk of Myocardial Infarction
Anna Helgadottir, *et al.*
Science **316**, 1491 (2007);
DOI: 10.1126/science.1142842

Science
AAAS

A Common Allele on Chromosome 9 Associated with Coronary Heart Disease
Ruth McPherson, *et al.*
Science **316**, 1488 (2007);
DOI: 10.1126/science.1142447

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 2, 2007 VOL. 357 NO. 5

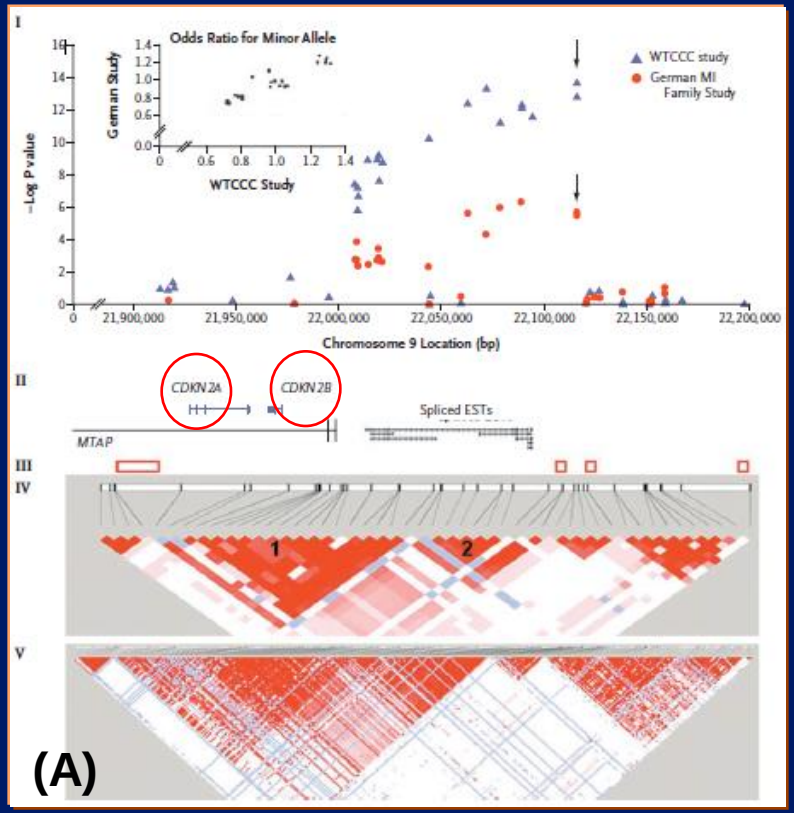
Genomewide Association Analysis of Coronary Artery Disease

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 2, 2007 VOL. 357 NO. 5

Genomewide Association Analysis of Coronary Artery Disease

Nilesh J. Samani, F.Med.Sci., Jeanette Erdmann, Ph.D., Alistair S. Hall, F.R.C.P., Christian Hengstenberg, M.D., Massimo Mangino, Ph.D., Bjoern Mayer, M.D., Richard J. Dixon, Ph.D., Thomas Meitinger, M.D., Peter Braund, M.Sc., H.-Erich Wichmann, M.D., Jennifer H. Barrett, Ph.D., Inke R. König, Ph.D., Suzanne E. Stevens, M.Sc., Silke Szymczak, M.Sc., David-Alexandre Tregouet, Ph.D., Mark M. Iles, Ph.D., Friedrich Pahlke, M.Sc., Helen Pollard, M.Sc., Wolfgang Lieb, M.D., Francois Cambien, M.D., Marcus Fischer, M.D., Willem Ouwehand, F.R.C.Path., Stefan Blankenberg, M.D., Anthony J. Balmforth, Ph.D., Andrea Baessler, M.D., Stephen G. Ball, F.R.C.P., Tim M. Strom, M.D., Ingrid Brænne, M.Sc., Christian Gieger, Ph.D., Panos Deloukas, Ph.D., Martin D. Tobin, M.F.P.H.M., Andreas Ziegler, Ph.D., John R. Thompson, Ph.D., and Heribert Schunkert, M.D., for the WTCCC and the Cardiogenics Consortium*



(A)

Table 2. Loci from the WTCCC Study with Significant Associations with Coronary Artery Disease That Were Replicated in the German MI Family Study.*

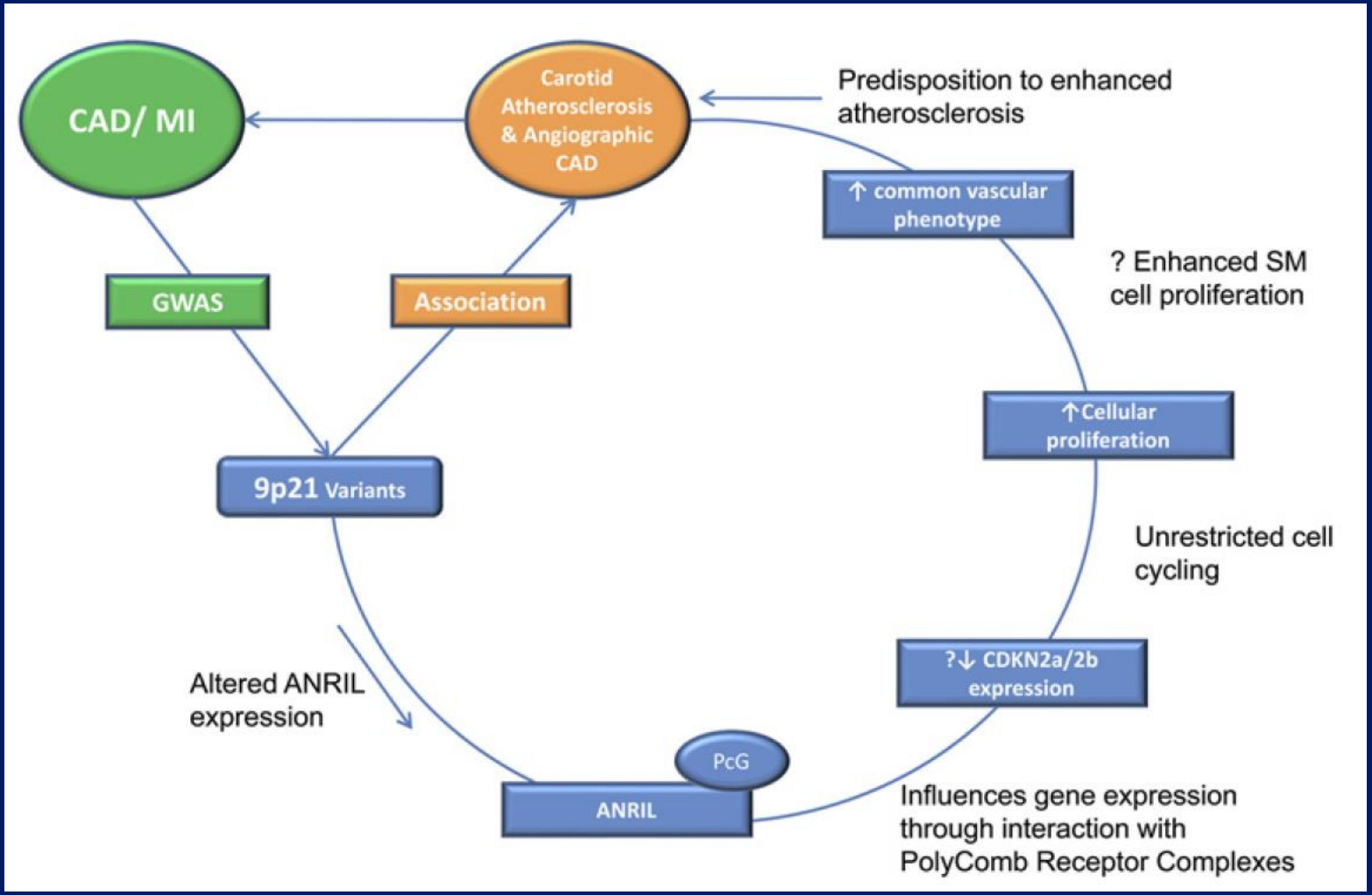
Chromosome	Lead SNP	Minor Allele in Controls	Risk Allele	Data	Frequency of Minor Allele		Odds Ratio for Risk Allele (95% CI)	Population Attributable Fraction	P Value
					Case Subjects	Controls			
2	rs2943634	A	C	WTCCC	0.30	0.34	1.22 (1.11–1.33)		1.19×10^{-5}
				German	0.32	0.37	1.20 (1.06–1.35)		0.004
				Adjusted German			1.08 (0.90–1.31)	0.10	0.03
6	rs6922269	A	A	WTCCC	0.29	0.25	1.23 (1.13–1.35)		6.33×10^{-6}
				German	0.30	0.26	1.24 (1.09–1.41)		0.001
				Adjusted German			1.23 (1.01–1.50)	0.11	0.009
9	rs1333049	C	C	WTCCC	0.55	0.47	1.37 (1.26–1.48)		1.80×10^{-14}
				German	0.54	0.48	1.33 (1.18–1.51)		6.80×10^{-6}
				Adjusted German			1.28 (1.07–1.53)	0.22	6.12×10^{-5}

(B)

Human chromosome 9p21.3 (rs1333049) had the strongest association with CAD in WTCCC and Germans

Samani NJ *et al*. NEJM 2007;357:443-453

Role of 9p21 in the pathogenesis of CAD

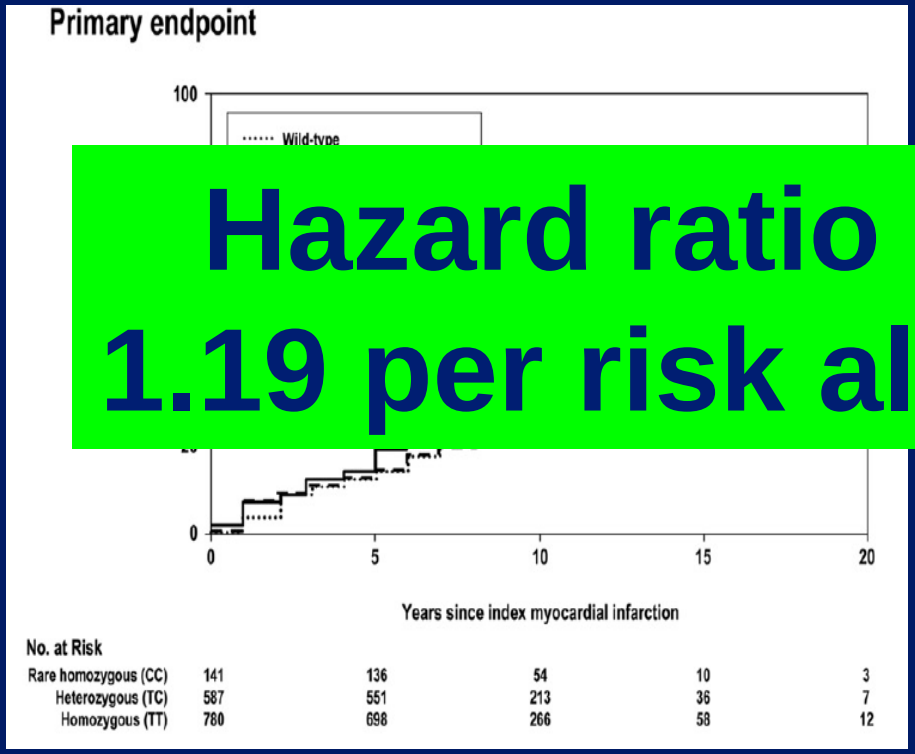


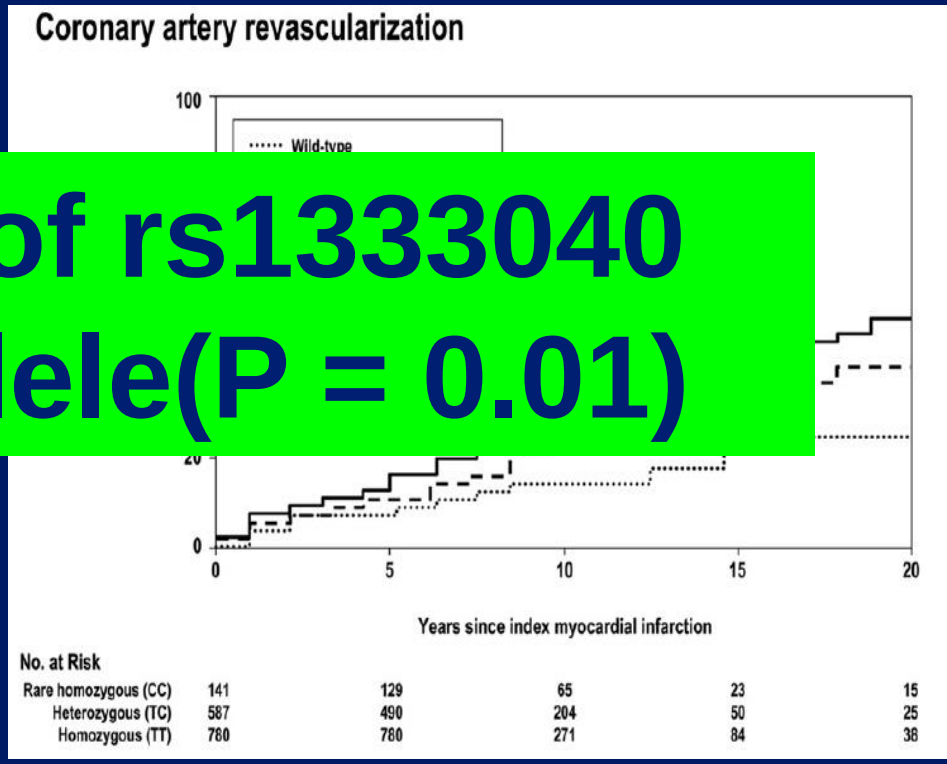
Patel RS *et al*. Heart 2011;97:1463-1473

Influence of 9p21.3 Genetic Variants on Clinical and Angiographic Outcomes in Early-Onset Myocardial Infarction

Ardissino D *et al*. J Am Coll Cardiol 2011;58:426-434

Hazard ratio of rs1333040 1.19 per risk allele(P = 0.01)

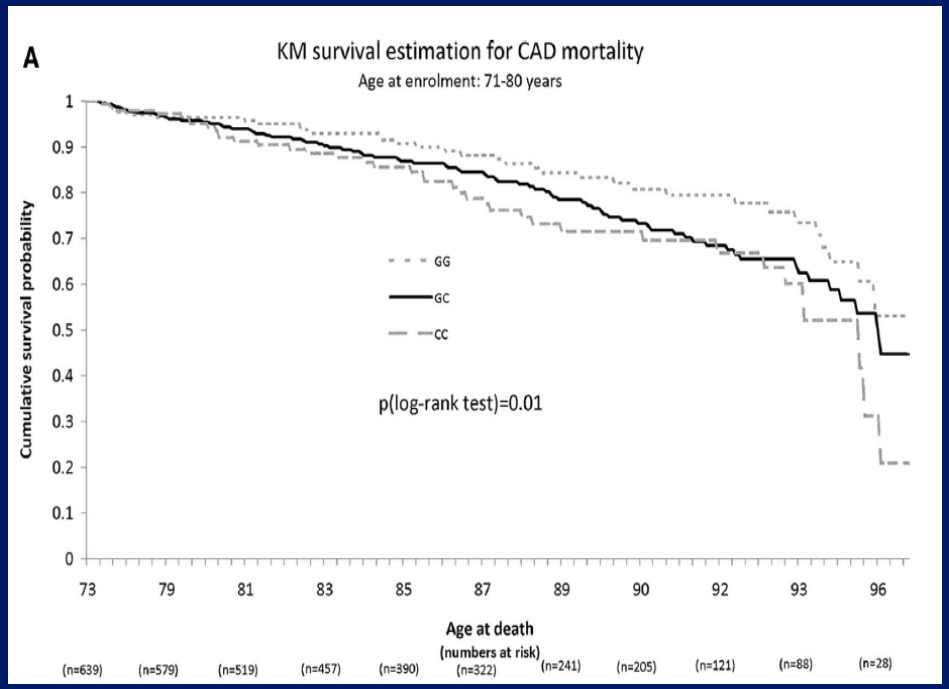




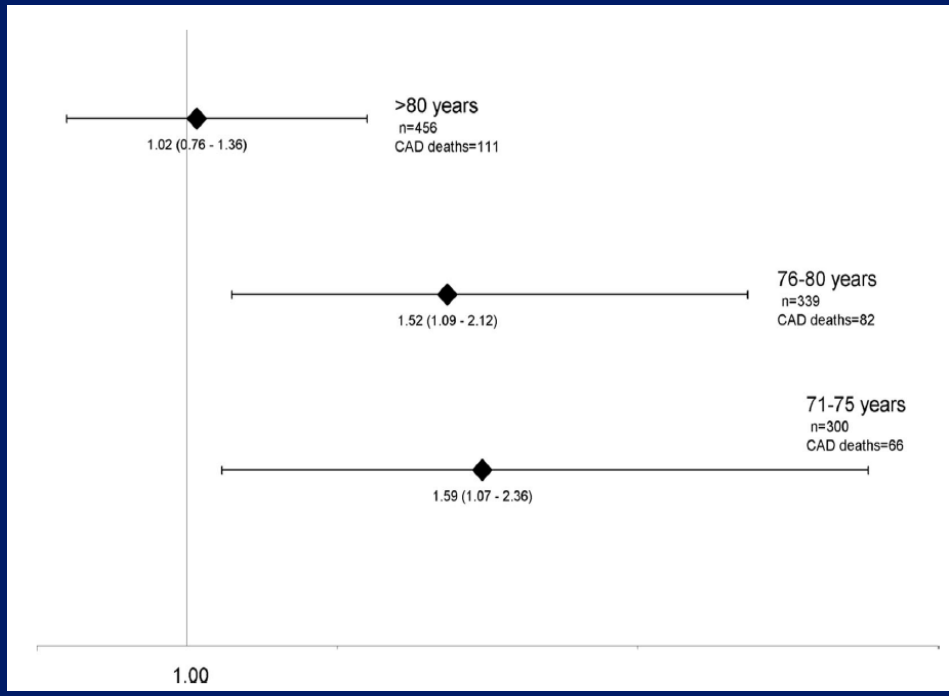
The Coronary Artery Disease–Associated 9p21 Variant and Later Life 20-Year Survival to Cohort Extinction

Ambarish Dutta, MBBS, MPH; William Henley, PhD; Iain A. Lang, PhD; Anna Murray, PhD; Jack Guralnik, MD, PhD; Robert B. Wallace, MD, MSc; David Melzer, MBBCh, PhD

Dutta A *et al*. Circ Cardiovasc Genet 2011;4:542-548



Survival estimation
According to presence of C allele



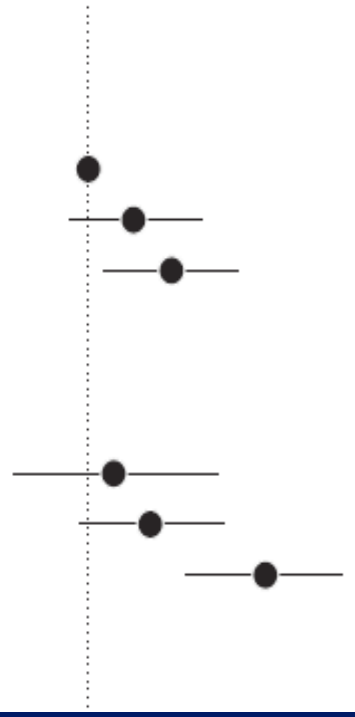
Hazard Ratio
According to presence of C allele

Interaction of 9p21 with diabetes and CVD

Case Control study of 734 type 2 Diabetics(322 CAD, 412 non CAD)

HbA_{1c} at Study Entry

	Coronary Artery Disease No. %	
Tertiles 1 and 2 (≤7.6%) rs2383206 genotype	Absent	Present
A/A	62 (21.2)	28 (13.9)
A/G	139 (47.6)	92 (45.5)
G/G	91 (31.2)	82 (40.6)
Tertile 3 (>7.6%) rs2383206 genotype		
A/A	23 (19.2)	13 (10.8)
A/G	69 (57.5)	53 (44.2)
G/G	28 (23.3)	54 (45.0)



Doria A *et al*. JAMA 2008;300(20)

nature genetics

Genome-wide association identifies a susceptibility locus for coronary artery disease in the Chinese Han population

Fan Wang[1,12], Cheng-Qi Xu[1,12], Qing He[2,12], Jian-Ping Cai[2,12], Xiu-Chun Li[1,12], Dan Wang[1,12], Xin Xiong[1,12], Yu-Hua Liao[3,12], Qiu-Tang Zeng[3,12], Yan-Zong Yang[4,12], Xiang Cheng[3,12], Cong Li[1], Rong Yang[1], Chu-Chu Wang[1], Gang Wu[5], Qiu-Lun Lu[1], Ying Bai[1], Yu-Feng Huang[1], Dan Yin[1], Qing Yang[1], Xiao-Jing Wang[1], Da-Peng Dai[2], Rong-Feng Zhang[4], Jing Wan[6], Jiang-Hua Ren[6], Si-Si Li[1], Yuan-Yuan Zhao[1], Fen-Fen Fu[1], Yuan Huang[1], Qing-Xian Li[7], Sheng-Wei Shi[7], Nan Lin[7], Zhen-Wei Pan[8], Yue Li[9], Bo Yu[10], Yan-Xia Wu[11], Yu-He Ke[11], Jian Lei[11], Nan Wang[1], Chun-Yan Luo[1], Li-Ying Ji[1], Lian-Jun Gao[4], Lei Li[1], Hui Liu[1], Er-Wen Huang[1], Jin Cui[1], Na Jia[2], Xiang Ren[1], Hui Li[1], Tie Ke[1], Xian-Qin Zhang[1], Jing-Yu Liu[1], Mu-Gen Liu[1], Hao Xia[5], Bo Yang[5], Li-Song Shi[1], Yun-Long Xia[4], Xin Tu[1] & Qing K Wang[1]

Identification of significant association between 2 SNPs and CAD in Chinese

Chr.	SNP	Gene (nearby)	Risk allele	OR	P	*vs.* KOR/EUR
6p24.1	rs6903956	*C6orf105*	A	1.71	$5.0X10^{-3}$	N
9p21.3	rs1333048	*CDKN2A/2B*	G	1.29	$4.0X10^{-3}$	Y

The function of *C6orf105* gene is unknown, but Wang et al. suggested that decreased expression of *C6orf105* gene may be a possible pathogenic cause of CAD.

Clin Chem Lab Med 2009;47(8):917–922 © 2009 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2009.215

Chromosome 9p21 polymorphism is associated with myocardial infarction but not with clinical outcome in Han Chinese

Table 4 Association of rs1333049 with clinical outcome after MI.

	GG (n=99)	*GC* (n=265)	*CC* (n=156)	p-Value
Treatment				
Primary PCI, %	78.8	80.0	85.3	0.314
Aspirin, %	90.9	94.3	91.7	0.410
ACEI or ARB, %	67.7	64.9	62.8	0.730
β-Blocker, %	45.5	49.1	48.7	0.822
Statins, %	54.5	56.2	51.6	0.657
Duration, months	28±18	30±17	29±17	0.685
Adverse events				
Rehospitalization, n	1±1	1±1	1±1	0.263
Death, %	2.0	1.1	2.6	0.537
Non-fatal MI, %	5.1	1.9	3.2	0.265
Combined MACE, %	48.8	49.7	51.2	0.940

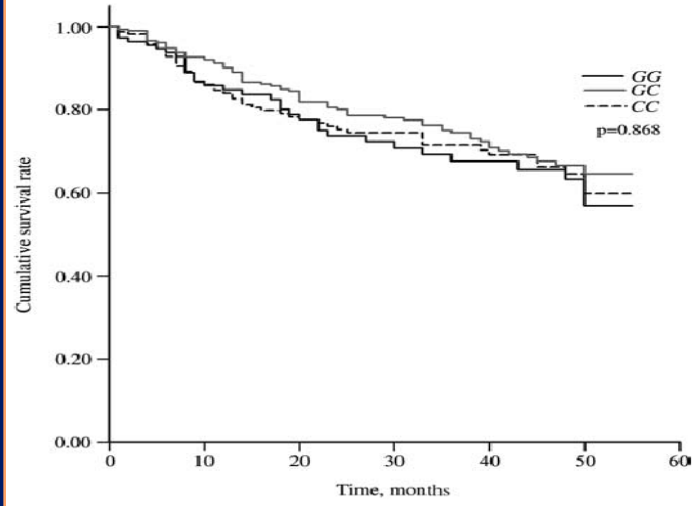


Figure 1 Kaplan-Meier survival plot by rs1333049. Cumulative survival rate was similar among the *GG* (n=99), *GC* (n=265), and *CC* (n=156) genotypes.

rs1333049 is associated with risk for mi, but not with post-MI prognosis in Han Chinese

Peng WH *et al*. CCLM 2009;47:917-22

Four SNPs on Chromosome 9p21 in a South Korean Population Implicate a Genetic Locus That Confers High Cross-Race Risk for Development of Coronary Artery Disease

Gong-Qing Shen, Lin Li, Shaoqi Rao, Kalil G. Abdullah, Ji Min Ban, Bok-Soo Lee, Jeong Euy Park, Qing K. Wang

Table 2. Allelic Association of Four SNPs on Chromosome 9p21 with CAD in a South Korean Population

SNP	Allele	Frequency		*P*-HW*	OR (95% CI)†	*P*-ob‡	*P*-adj§	*P*-emp¶
		Control	Case					
rs10757274	**G**	0.439	0.503	0.37	1.29 (1.06–1.58)	0.011	0.010	0.013
rs2383206	G	0.441	0.506	0.88	1.30 (1.06–1.58)	0.011	0.024	0.011
rs2383207	G	0.647	0.707	0.59	1.32 (1.06–1.63)	0.011	0.001	0.011
rs10757278	G	0.457	0.521	0.80	1.29 (1.06–1.57)	0.011	0.001	0.013

*P-HW, *P* value for Hardy-Weinberg disequilibrium analysis. †OR, odds ratio, CI, confidence interval. ‡*P*-obs, uncorrected *P* value. §*P*-adj, *P* value obtained after adjustment for gender, age, hypertension, and diabetes. ¶*P*-emp, permutation *P* value calculated using 100 000 Monte Carlo simulations.

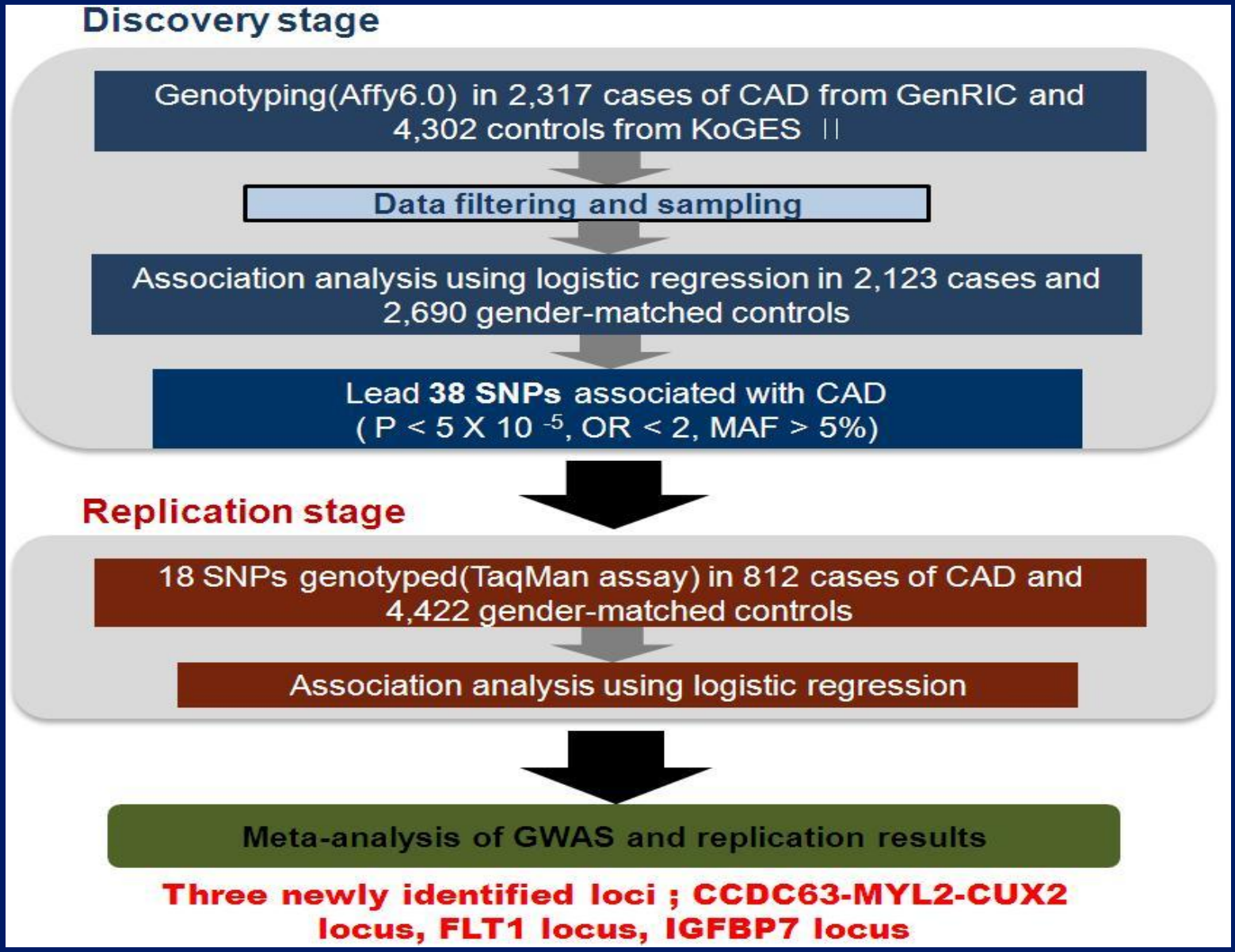
Table. Association between haploypes with CAD in Koreans

Haplotype	Control (%)	Case (%)	OR (95% CI)*	*P*-obs†	*P*-emp‡
GGGG	42.2	48.1	1.28 (1.04–1.56)	0.017	0.019
AAAA	35.3	29.2	0.75 (0.60–0.92)	0.007	0.007
AAGA	16.8	15.9	0.93 (0.71–1.22)	0.612	0.633
AAGG	2.5	2.8	1.16 (0.62–2.17)	0.651	0.754
GAGA	1.1	1.3	1.27 (0.49–3.26)	0.619	0.657
AGGA	1.0	1.1	1.33 (0.48–3.72)	0.583	0.634

This study provided solid evidence that the 4 SNPs on 9p21 are associated with CAD in a South Koreans

Shon GQ *et al*. ATVB 2008;28

Schematic Overview of Identification of CAD Causative SNPs by GWAS [GenRIC Study]



Results of a meta-analysis for SNPs identified from both the GWAS and the replication cohorts

				GWAS - Korea				Replication - Japan				Combined analysis		
SNP	Chromosome	Gene	Func	Allele	N	OR	P	Allele	N	OR	P	OR	p	het.(*P*)
Previous publications														
rs4537545	**1q21.3e**	***IL6R***	**i**	**T**	**4735**	**0.8356**	**2.39E-05**	**T**	**5234**	**0.8936**	**0.04297**	**0.8659**	**4.74E-05**	**0.3376**
rs7588415	**2p24.1c**	***APOB***		**A**	**4778**	**0.7578**	**2.74E-05**	**A**	**5233**	**0.8232**	**0.02587**	**0.7914**	**2.47E-05**	**0.4498**
rs1333049	**9p21.3c**			**C**	**4770**	**1.263**	**3.30E-08**	**C**	**5000**	**1.47**	**1.8E-14**			
New identified loci														
rs1111782	9p21.2a	*TEK*	i	A	4716	0.8101	7.32E-07	T	5234	0.952	0.3684	0.8816	3.46E-04	0.0198
rs12114277	8q22.3b	*UBR5*	i	A	4674	0.8082	8.43E-07	A	5233	1.016	0.764	0.912	8.70E-03	9.00E-04
rs219822	7q22.1a	*TRRAP*	i	A	4783	1.229	9.64E-07	A	5232	1.068	0.2277	1.1422	1.35E-04	4.12E-02
rs12705702	7q31.1b			T	4781	0.8263	4.17E-06	G	5232	1.039	0.4792	0.9314	4.06E-02	8.00E-04
rs1163072	10q24.33			T	4780	1.203	1.04E-05	G	5231	1.051	0.3571	1.1208	9.86E-04	0.0487
rs41391154	3p26.1a	*GRM7*	i	T	4775	0.7166	1.24E-05	T	5233	0.9898	0.9076	0.8487	5.05E-03	0.0055
rs886126	12q24.11d	*CUX2*	i	C	4756	0.8244	1.31E-05	C	5232	0.9654	5.45E-01	0.8958	2.92E-03	0.0309
rs10012505	4q34.1b	*GALNT17*	i	G	4662	0.7661	1.67E-05	C	5233	0.9422	0.4594	0.8547	2.35E-03	0.0416
rs2122149	4q13.1a			A	4674	1.277	1.87E-05	A	5231	1.03	0.6516	1.14	2.96E-03	0.0138
rs9944810	18q21.31	*ALPK2*	cn	C	4783	0.8326	2.08E-05	C	5234	0.9488	0.3364	0.8914	1.09E-03	0.0603
SNP1	**12**	***MYL2***	**i**	**C**	**4762**	**1.255**	**2.13E-05**	**G**	**5232**	**1.262**	**9.85E-05**	**1.2586**	**1.13E-08**	**0.9446**
SNP2	**13**	***FLT1***	**i**	**C**	**4789**	**1.192**	**2.34E-05**	**G**	**5231**	**1.148**	**1.09E-02**	**1.1688**	**6.35E-06**	**0.5817**
SNP3	**4**	***IGFBP7***	**i**	**G**	**4781**	**1.187**	**3.27E-05**	**C**	**5233**	**1.116**	**0.04156**	**1.1491**	**4.91E-05**	**0.3625**

Hum Mol Genet [IF 8.058] Submitted

J Hum Genet (2008) 53:357–359
DOI 10.1007/s10038-008-0248-4

SHORT COMMUNICATION

Replication of the association between a chromosome 9p21 polymorphism and coronary artery disease in Japanese and Korean populations

Kunihiko Hinohara · Toshiaki Nakajima · Megumi Takahashi · Shigeru Hohda · Taishi Sasaoka · Ken-ichi Nakahara · Kouji Chida · Motoji Sawabe · Takuro Arimura · Akinori Sato · Bok-Soo Lee · Ji-min Ban · Michio Yasunami · Jeong-Euy Park · Toru Izumi · Akinori Kimura

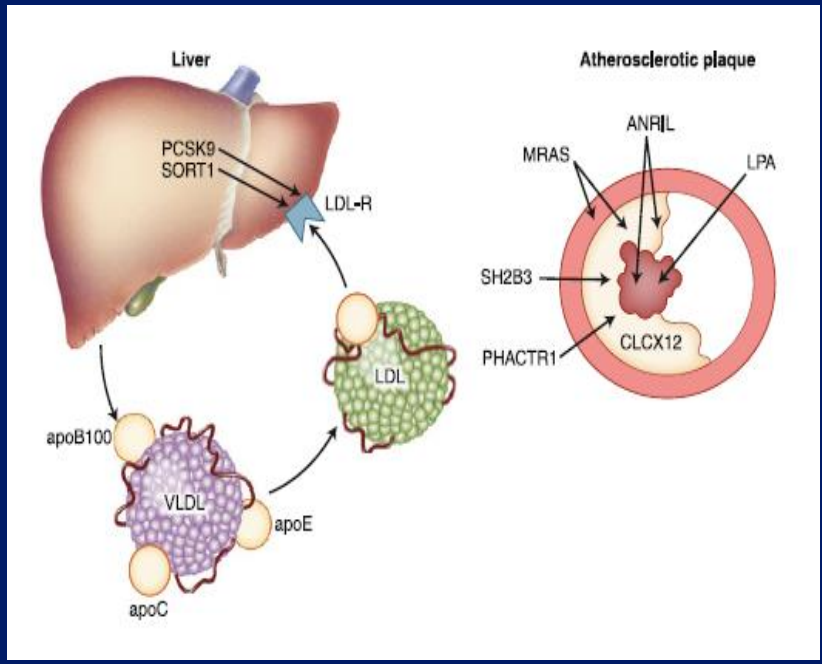
Association of rs1333049 on chromosome 9p21 with CAD in Japanese and Koreans

Genotype	CAD (n = 604) n (%)	Control (n = 1,151) n (%)	OR (95%CI)	p value
(a) Japanese				
GG	114 (18.9)	286 (24.9)	0.70 (0.55–0.90)	0.0046
GC	312 (51.7)	606 (52.7)	0.96 (0.79–1.17)	ns
CC	178 (29.5)	259 (22.5)	1.44 (1.15–1.80)	0.0013
C allele frequency	0.55	0.49	1.30 (1.13–1.49)	0.00027
HWE (p)	0.54	0.19		
Genotype	CAD (n = 679) n (%)	Control (n = 706) n (%)	OR (95%CI)	p value
(b) Korean				
GG	158 (23.3)	192 (27.2)	0.81 (0.64–1.04)	ns
GC	335 (49.3)	353 (50.0)	0.97 (0.79–1.20)	ns
CC	186 (27.4)	161 (22.8)	1.28 (1.00–1.63)	0.049
C allele frequency	0.52	0.48	1.19 (1.02–1.38)	0.025
HWE (p)	0.96	1.00		

Chr.9p21 rs1333049 was the susceptibility locus for CAD in Japanese and Koreans

17 Genetic Loci associated with CAD

Loci	Chromosomal location	SNP	RAF, %	Odds ratio per risk allele (95% CI)	Candidate genes	Putative mechanism
1	1p13	rs599839	77	1.13 (1.08–1.19)	*CELSR2, PSCR1, SORT1*	LDL mediated
	1p13	rs646776	81	1.19 (1.13–1.26)	*CELSR2, PSCR1, SORT1*	
2	2p32	rs11206510	81	1.15 (1.10–1.210	*PCSK9*	LDL mediated
3	1q41	rs3008621	72	1.10 (1.04–1.17)	*MIA3*	Unknown
	1q41	rs17465637	72	1.14 (1.10–1.19)	*MIA3*	
4	2q33	rs6725887	14	1.17 (1.11–1.19)	*WDRI2*	Unknown
5	2q36	rs2972416	37	0.46	*IRS1*	Defective insulin signaling and NO production
6	3q22	rs9818870	15	1.15 (1.11–1.19)	*MRAS*	Adhesion signaling
7	6p21.31	rs2814982	16	0.49	*C6orf106*	Unknown
8	6p24	rs12526453	65	1.12 (1.08–1.17)	*PHACTR1*	Coronary calcification
9	6q26–6q27	rs2048327	18	1.20 (1.13–1.28)	*LPA,LPAL2, SLC22A3*	Promotes atherothrombosis
	6q26–6q27	rs3127599	18	1.20 (1.13–1.28)	*LPA,LPAL2, SLC22A3*	
	6q26–6q27	rs7767084	18	1.20 (1.13–1.28)	*LPA,LPAL2, SLC22A3*	
	6q26–6q27	rs10755578	18	1.20 (1.13–1.28)	*LPA,LPAL2, SLC22A3*	
	6q26–6q27	rs3798220	2	1.47 (1.35–1.60)	*LPA,LPAL2, SLC22A3*	
	6q26–6q27	rs10455872	7	1.68 (1.43–1.98)	*LPA,LPAL2, SLC22A3*	
10	7q32	rs4731702	48	0.59	*KLF14*	Unknown
11	8p22	rs1495741	22	2.85	*NAT2*	Unknown
12	9p21	rs1333049	52	1.20 (1.16–1.25)	*ANRIL, CDKN2A, CDKN2B, MTAP*	Increased proliferation of smooth muscle cells
	9p21	rs4977574	56	1.29 (1.25–1.34)	*ANRIL, CDKN2A, CDKN2B, MTAP*	
	9p21	rs10757274	48		*ANRIL, CDKN2A, CDKN2B, MTAP*	
	9p21	rs28383206	51		*ANRIL, CDKN2A, CDKN2B, MTAP*	
	9p21	rs2383207	51	1.22 (1.13–1.33)	*ANRIL, CDKN2A, CDKN2B, MTAP*	
	9p21	rs107572378	47	1.25 (1.15–1.36)	*ANRIL, CDKN2A, CDKN2B, MTAP*	
	9p21	rs10116277	48		*ANRIL, CDKN2A, CDKN2B, MTAP*	
13	10q11	rs501120	84	1.11 (1.05–1.18)	*CXCL12*	Neointima formation after arterial injury, platelet activation in atherosclerotic lesions
	10q11	rs1746048	84	1.17 (1.11–1.24)	*CXCL12*	
14	12q24	rs2259816	37	1.08 (1.05–1.11)	*HNF1A*	apoM-mediated HDL modification
15	12q24	rs3184504	40	1.13 (1.11–1.19)	*SH2B3*	Reduced anti-inflammatory activity contributes to the progression of plaques
	12q24	rs11065987	34	1.14 (1.10–1.19)	*SH2B3*	
16	16p13	rs1122608	75	1.15 (1.10–1.21)	*LDLR*	LDL-mediated
17	21q22	rs9882601	13	1.20 (1.14–1.27)	*KCNHE2, MRPS6, SLC5A3*	Unknown



Sivapalaratnam S *et al*. Curr Atheroscler Rep 2011;13:225-232

Thirty-five common variants for coronary artery disease: the fruits of much collaborative labour

John F. Peden and Martin Farrall*

Department of Cardiovascular Medicine, The Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

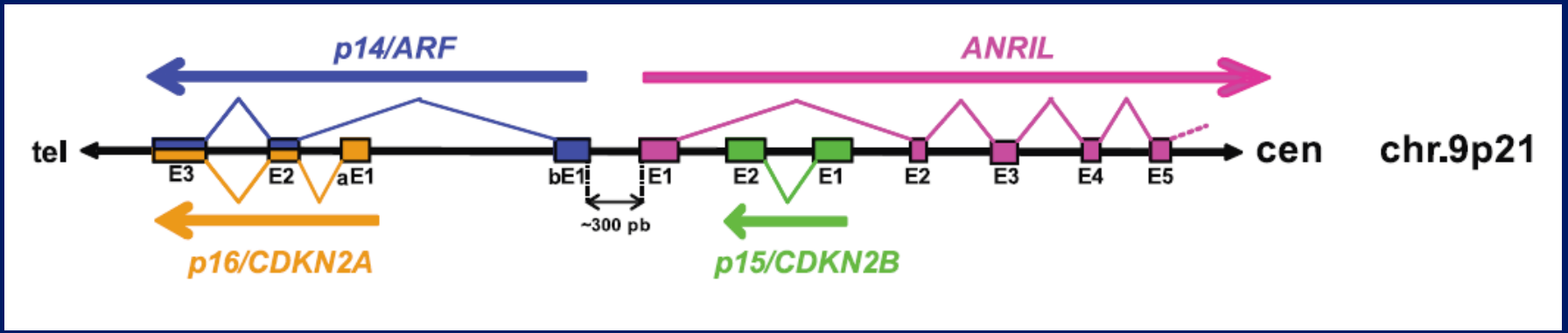
Hum Mol Genet 2011;20:R198

Chr	Position	Locus[a]	SNP	References	Reported effect EAF	OR	SNP-specific heritability (h^2_{SNP}), % K_p= 2%	K_p= 5%	K_p= 10%
1	55 496 039	PCSK9	rs11206510	MIGen (36)	0.82	1.08	0.03	0.04	0.05
1	56 962 821	PPAP2B	rs17114036	CARDIoGRAM (3)	0.91	1.17	0.07	0.09	0.11
1	109 822 166	SORT1	rs599839	Samani *et al.* (58), MIGen (36)	0.78	1.11	0.06	0.08	0.10
1	222 823 529	MIA3	rs17465637	Samani *et al.* (58), MIGen (36)	0.74	1.14	0.12	0.15	0.18
2	44 072 576	ABCG8	rs4299376	HumanCVD (8)	0.29	1.09	0.04	0.05	0.07
2	203 745 885	WDR12	rs6725887	MIGen (36)	0.15	1.14	0.07	0.09	0.11
3	138 119 952	MRAS	rs2306374	Erdmann *et al.* (59)	0.18	1.12	0.06	0.07	0.09
5	131 867 702	IL5	rs2706399	HumanCVD (8)	0.48	1.02	0.01	0.01	0.01
6	11 774 583	C6orf105	rs6903956[b]	Wang *et al.* (7)	0.07	1.51	0.35	0.45	0.56
6	12 927 544	PHACTR1	rs12526453	MIGen (36)	0.67	1.10	0.06	0.08	0.10
6	35 034 800	ANKS1A	rs17609940	CARDIoGRAM (3)	0.75	1.07	0.03	0.04	0.05
6	134 214 525	TCF21	rs12190287	CARDIoGRAM (3)	0.62	1.08	0.05	0.06	0.07
6	160 961 137	LPA	rs3798220	Clarke *et al.* (30)	0.02	1.92	0.25	0.32	0.40
6	161 010 118	LPA	rs10455872	Clarke *et al.* (30)	0.07	1.70	0.57	0.73	0.90
7	107 244 545	7q22	rs10953541	C4D 2011 (4)	0.80	1.08	0.05	0.06	0.08
7	129 663 496	ZC3HC1	rs11556924	CARDIoGRAM (3)	0.62	1.09	0.06	0.07	0.09
8	126 495 818	TRIB1	rs10808546	HumanCVD (8)	0.65	1.04	0.02	0.02	0.02
9	22 098 574	ANRIL/CDKN2BAS	rs4977574	WTCCC (60), McPherson *et al.* (61), Helgadottir *et al.* (62), Samani *et al.* (58), MIGen (36)	0.46	1.29	0.53	0.68	0.84
9	136 154 168	ABO	rs579459	CARDIoGRAM (3), Reilly *et al.* (6)	0.21	1.10	0.05	0.06	0.08
10	30 335 122	KIAA1462	rs2505083	C4D 2011 (4), Erdmann *et al.* (5)	0.38	1.07	0.05	0.06	0.08
10	44 775 824	CXCL12	rs1746048	Samani *et al.* (58), MIGen (36)	0.87	1.09	0.03	0.03	0.04
10	91 002 927	LIPA	rs1412444	C4D 2011 (4)	0.42	1.08	0.05	0.07	0.08
10	104 719 096	CYP17A1-NT5C2	rs12413409	CARDIoGRAM (3)	0.89	1.12	0.04	0.05	0.07
11	103 660 567	PDGFD	rs974819	C4D 2011 (4)	0.32	1.08	0.05	0.06	0.08
11	116 648 917	APOA1-C3-A4-A5	rs964184	CARDIoGRAM (3)	0.13	1.13	0.05	0.07	0.09
12	111 884 608	SH2B3	rs3184504	Soranzo *et al.* (63)	0.44	1.07	0.04	0.05	0.06
13	110 960 712	COL4A1-A2	rs4773144	CARDIoGRAM (3)	0.44	1.07	0.04	0.05	0.06
14	100 133 942	HHIPL1	rs2895811	CARDIoGRAM (3)	0.43	1.07	0.04	0.05	0.06
15	79 111 093	ADAMTS7	rs4380028	C4D 2011 (4), CARDIoGRAM (3), Reilly *et al.* (6)	0.60	1.07	0.05	0.06	0.08
17	2 126 504	SMG6-SRR	rs216172	CARDIoGRAM (3)	0.37	1.07	0.03	0.05	0.06
17	17 543 722	PEMT	rs12936587	CARDIoGRAM (3)	0.56	1.07	0.04	0.05	0.06
17	46 988 597	GIP-ATP	rs46522	CARDIoGRAM (3)	0.53	1.06	0.03	0.04	0.04
19	11 163 601	LDLR	rs1122608	MIGen (36)	0.77	1.14	0.10	0.12	0.15
19	45 395 619	APOE	rs2075650	HumanCVD (8)	0.14	1.14	0.07	0.09	0.11
21	35 599 128	MRPS6	rs9982601	MIGen (36)	0.15	1.18	0.11	0.14	0.18
h^2_{total}							**3.30**	**4.27**	**5.29**

ANRIL, a long, noncoding RNA, is an unexpected major hotspot in GWAS

Eric Pasmant,*,†,[1] Audrey Sabbagh,*,† Michel Vidaud,*,† and Ivan Bièche*,†

*Unité Mixte de Recherche (UMR)745 Institut National de la Santé et de la Recherche Médicale (INSERM), Université Paris Descartes, Faculté des Sciences Pharmaceutiques et Biologiques, Paris, France; and †Service de Biochimie et Génétique Moléculaire, Hôpital Beaujon, Clichy, France

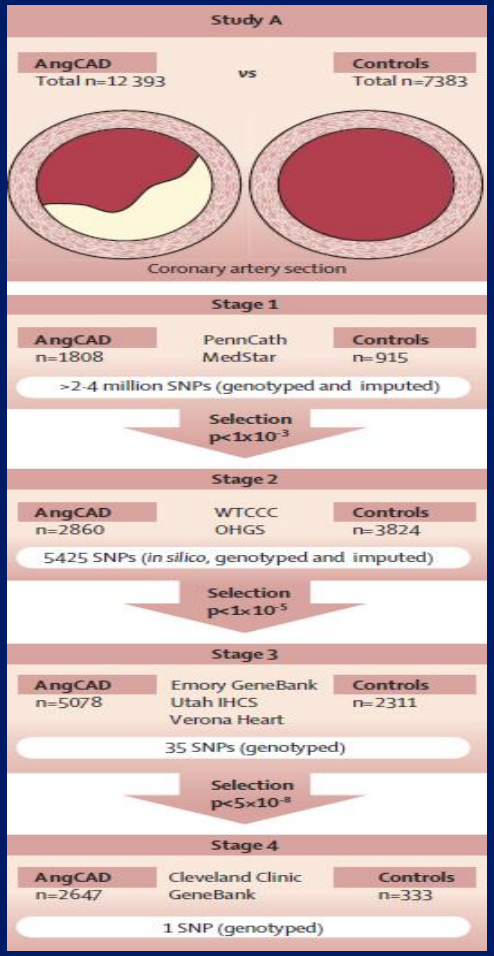


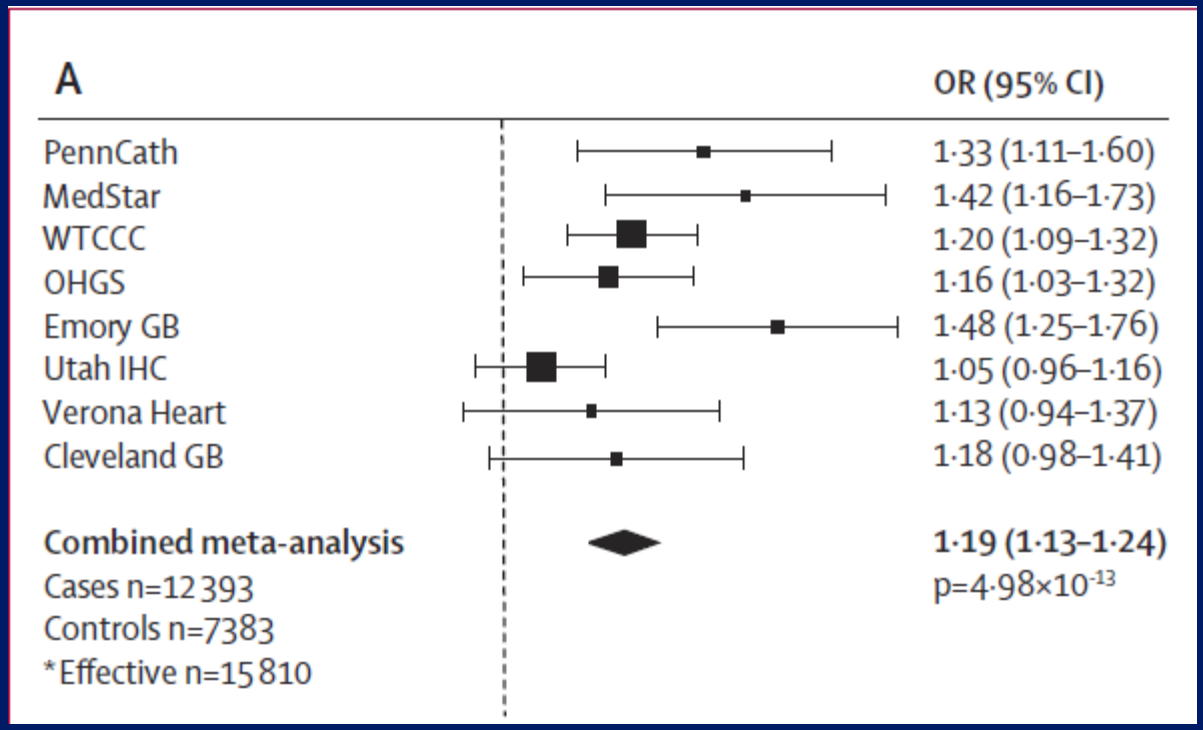
ANRIL important for expression of CDK activity and vascular proliferation

Pasmant E *et al*. FASEB J 2011;25:444-448

Identification of *ADAMTS7* as a novel locus for coronary atherosclerosis and association of *ABO* with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies

Reilly M *et al*. Lancet 2011;377:383-392

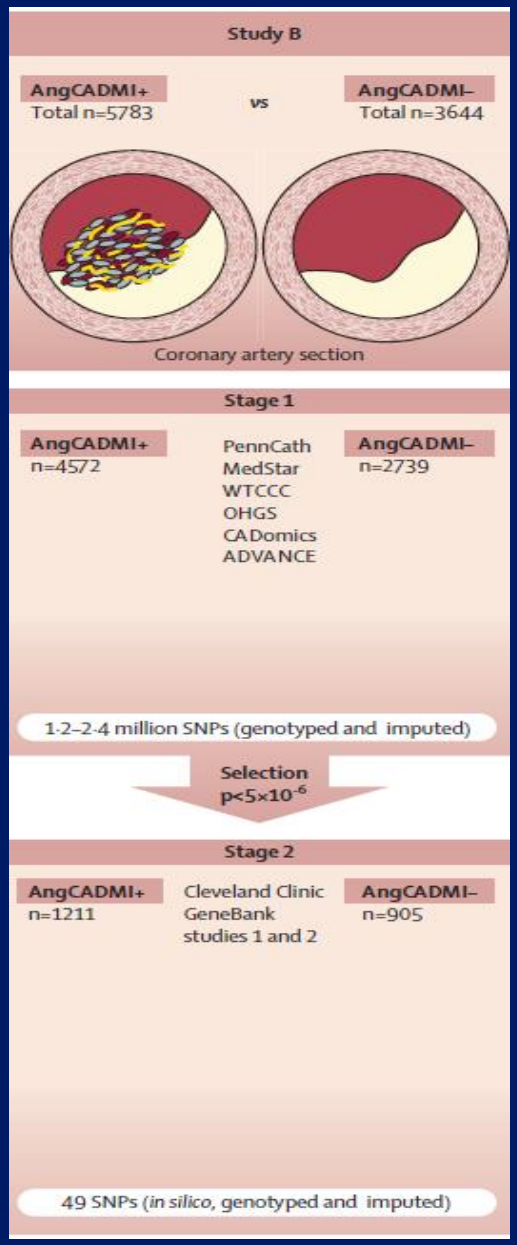


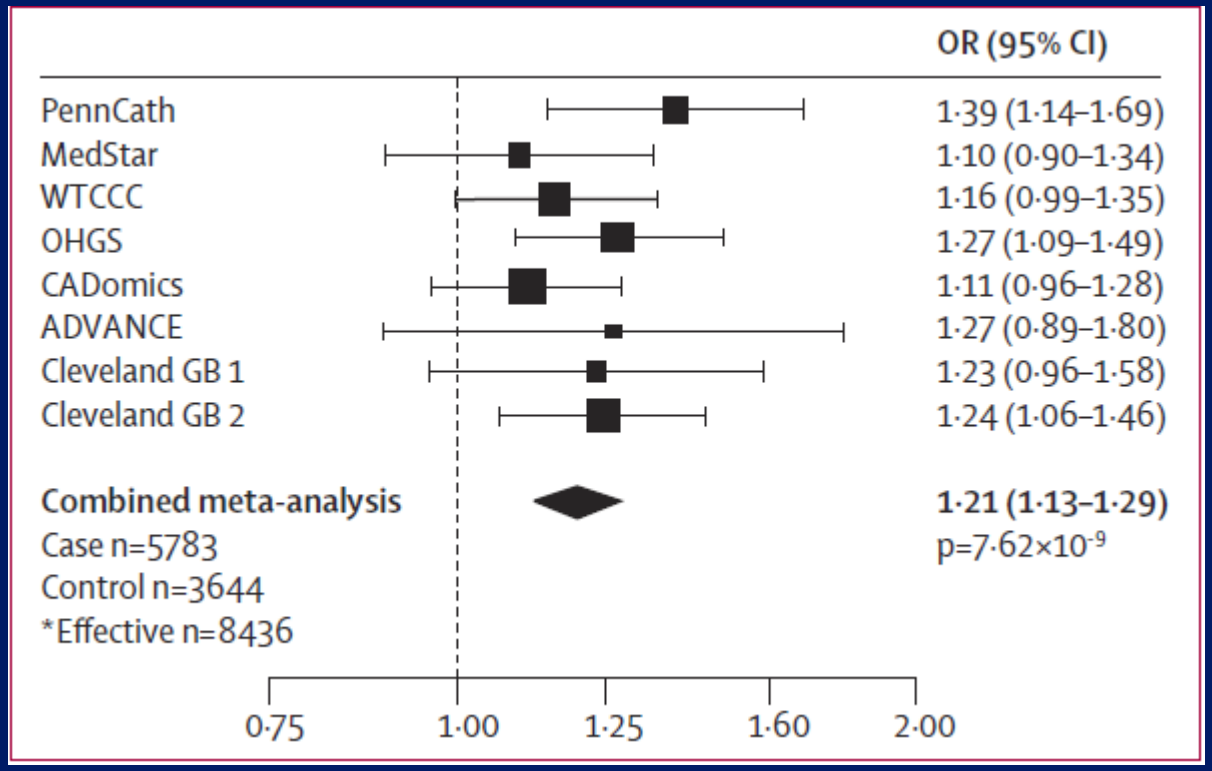


Odds Ratio of CAD According to Presence of ADAMTS7 variant

rs514659 at 9q34.2 at ABO locus and MI

Reilly M *et al*. Lancet 2011;377:383-392





Odds Ratio of MI According to Presence of rs514659 variant at ABO

GWAS of CAD in CGC study

Genome-wide association study (CAD: control=230:290)
CAD – multivesseles dx age<55

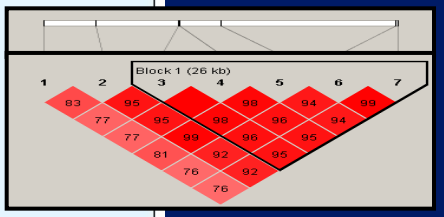
Affymetrix GeneChip Mapping 500K Array Set analysis

Significance SNP p<0.001 #2187
Including surrounding SNP, cSNP

2nd Validation Study (CAD:control = 1172:1087)
CAD – singlevessele dx age<65

Affymetrix Targeted GeneChip 3K array analysis

Significance SNP p<0.001 **#38**
Including significance SNP in Japanese



3rd Validation study [CGC Cohort]
(CAD : control = 502:648)

Confirmation of Korean specific SNPs

Cumulative association analysis

4th Validation study (CAD progress)

Identify significant SNPs

Clinical follow up study

Construction of CAD-related CGC Cohort

CAD group (503 명)
- Male: ≥55 years, Female: ≥60 years
- Multi-vessels

Control group (503 명)
- Case group과 1:1 matching
- Control 146명 추가 분석

Conventional Factors		Biomarkers	SNP (32)			
Age	BUN	ADIPOQ	rs1801133	rs2569190	rs1333049	rs4341
Sex	Creatinine	LP-PLA_2	rs17465637	rs10946398	rs2230806	rs1502017
DM	Glucose	RAGE	rs12713259	rs909253	rs4149263	
HT	Insulin	IL-6	rs16944	rs2070600	rs13290387	
Smoke	hsCRP	RANTES	rs1143623	rs1051931	rs501120	
BMI			rs4848306	rs16874954	rs5015480	
T-Chol			rs4402960	rs1871388	rs5215	
HDL			rs2241766	rs1800796	rs11048979	
TG			rs1501299	rs13266634	rs8050136	
LDL			rs155948	rs564398	rs2107538	

Results of CAD-GWAS in CGC

			GWAS	Repl. 1	Repl. 2	Repl. 3
		Case / Control	230/290	1392/1355	502/648	2123/2690
		Mean age(case)	48.3±4.72	54.5 ±7.79	49.3 ±5.36	51.6±7.52
		Age criteria	≤55	≤65	≤55(m), 60(f)	< 55(m), 60(f)
Chr.	Gene	rs #	Stage1 (500K)	Stage2 (3K)	Stage3 (32)	Stage4 (15)
9p		rs133****	5.643E-05	3.080E-07	0.0109	3.30E-08
2p	SPTBN1	rs127****	3.009E-06	1.060E-05	0.0048	0.0034
5q		rs15****	3.125E-03	1.770E-05	0.0049	0.0455
12p	ARNTL2	rs1104****	8.994E-04	6.920E-06	0.0047	0.5961
19p	CACNA1A	rs150****	4.839E-05	4.320E-05	0.0890	-
1p	Vav3	rs1275****	6.192E-03	1.906E-03	0.3889	-
1p		rs599***	0.0272	-	0.8887	-
1q	MIA3	rs1746****	0.0860	-	0.0227	0.0934
10q		rs501***	0.9211	-	0.0100	-

Genome-wide association analysis and replication of coronary artery disease in South Korea suggests a causal variant common to diverse populations

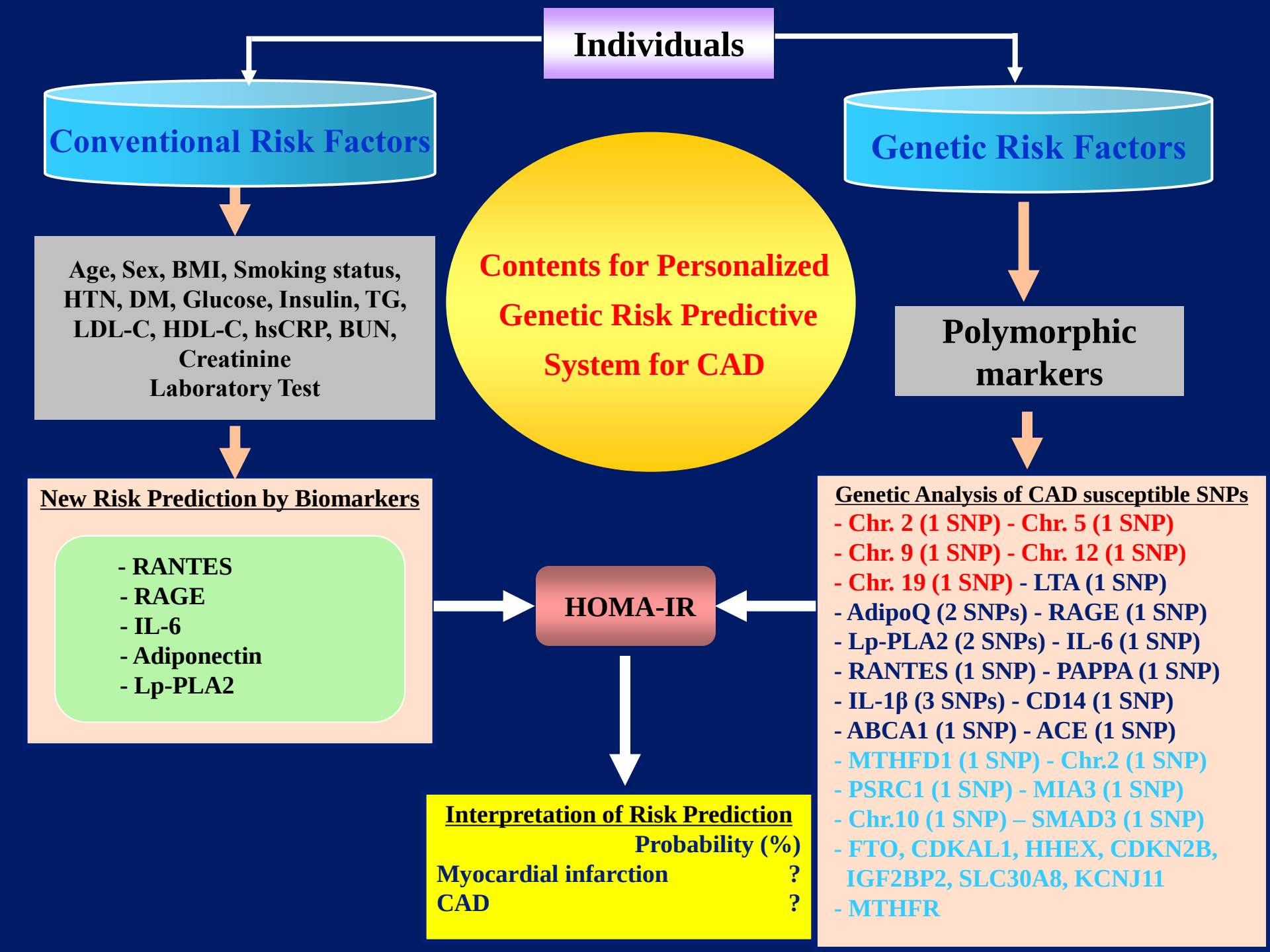
Eun Young Cho,[2] Yangsoo Jang,[3] Eun Soon Shin,[2] Hye Yoon Jang,[2] Yeon-Kyeong Yoo,[2] Sook Kim,[2] Ji Hyun Jang,[2] Ji Yeon Lee,[2] Min Hye Yun,[2] Min Young Park,[2] Jey Sook Chae,[3] Jin Woo Lim,[4] Dong Jik Shin,[4] Sungha Park,[4] Jong Ho Lee,[3] Bok Ghee Han,[5] Kim Hyung Rae,[5] Lon R Cardon,[6] Andrew P Morris,[1] Jong Eun Lee,[2] Geraldine M Clarke[1]

Table 2 Stage 1, Stage 2 and combined Stage 1 and Stage 2 samples association results for risk of coronary artery disease at single nucleotide polymorphisms in 9p21 with p<1e—04 in the combined sample

Single nucleotide polymorphism	Allele		Stage	Genotypes minor homozygous/ heterozygous/major homozygous		Minor allele frequency		OR for risk allele (95% CI)	p Value
	Minor	Risk		Control	Case	Control	Case		
rs6475606	C	T	S1	24/124/120	13/80/132	0.32	0.24	1.55 (1.16 to 2.07)	3.24e—03
			S2	123/450/474	98/458/580	0.33	0.29	1.22 (1.08 to 1.39)	2.00e—03
			S1&S2	147/574/594	111/538/712	0.33	0.28	1.27 (1.13 to 1.43)	5.75e—05
rs4977574	G	G	S1	44/133/91	63/117/45	0.41	0.54	1.71 (1.31 to 2.22)	6.74e—05
			S2	199/522/345	274/569/315	0.43	0.48	1.22 (1.08 to 1.37)	1.27e—03
			S1&S2	243/655/436	337/686/360	0.43	0.49	1.29 (1.16 to 1.44)	4.61e—06
rs2891168	G	G	S1	43/134/91	63/116/45	0.41	0.54	1.73 (1.32 to 2.25)	5.16e—05
			S2	199/524/351	274/569/316	0.43	0.48	1.23 (1.09 to 1.38)	7.59e—04
			S1&S2	242/658/442	337/685/361	0.43	0.49	1.3 (1.17 to 1.45)	2.27e—06
rs1333042	A	G	S1	27/130/106	14/90/119	0.35	0.26	1.53 (1.15 to 2.04)	3.82e—03
			S2	156/473/455	122/490/555	0.36	0.31	1.23 (1.09 to 1.39)	1.04e—03
			S1&S2	183/603/561	136/580/674	0.36	0.31	1.27 (1.13 to 1.42)	3.71e—05
rs1333048	C	C	S1	55/134/76	71/119/35	0.46	0.58	1.68 (1.29 to 2.19)	1.38e—04
			S2	232/515/326	310/574/273	0.46	0.52	1.26 (1.12 to 1.42)	1.28e—04
			S1&S2	287/649/402	381/693/308	0.46	0.53	1.32 (1.18 to 1.47)	4.95e—07
rs1333049	C	C	S1	54/137/76	72/118/33	0.46	0.59	1.75 (1.34 to 2.29)	4.51e—05
			S2	232/519/334	309/574/282	0.45	0.51	1.25 (1.12 to 1.41)	1.50e—04
			S1&S2	286/656/410	381/692/315	0.45	0.52	1.32 (1.19 to 1.47)	3.08e—07

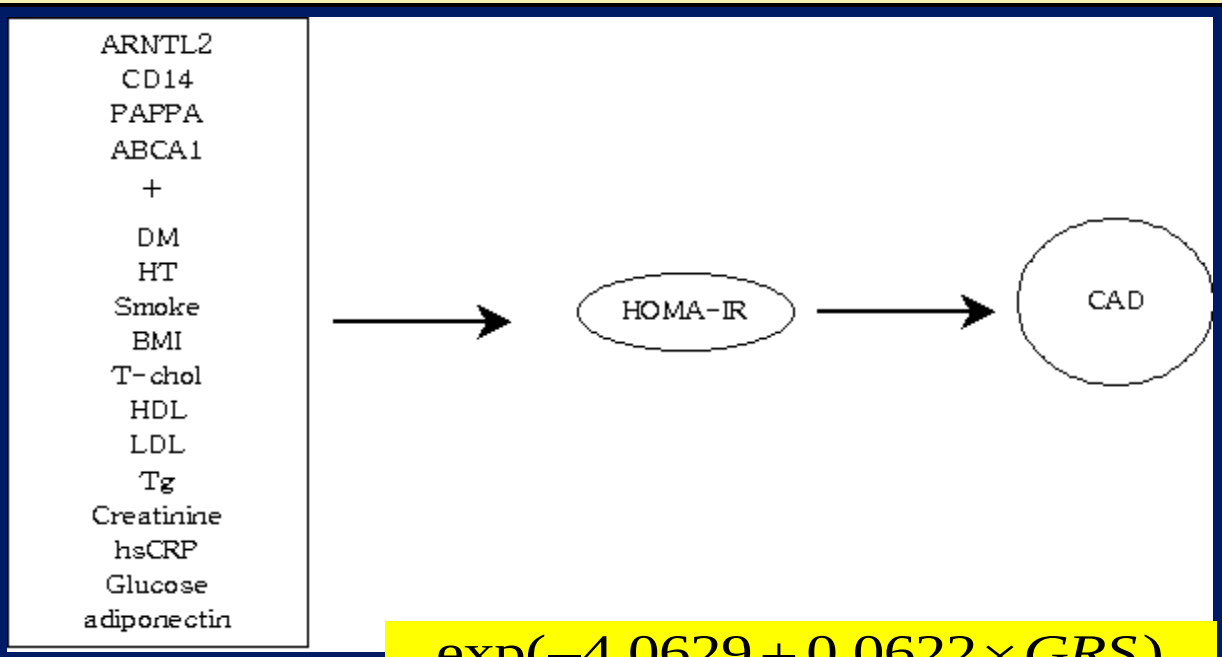
The most notable association with CAD was observed on chromosome 9p21.3.
The strongest signal was at rs1333049.
These results replicate signals first observed in Caucasians and subsequently observed in a variety of Asians including Japanese, Korean and Chinese Han.

Cho E *et al*. Heart Asia 2010;104-108


Individuals
Conventional Risk Factors
Genetic Risk Factors
Contents for Personalized Genetic Risk Predictive System for CAD
Age, Sex, BMI, Smoking status, HTN, DM, Glucose, Insulin, TG, LDL-C, HDL-C, hsCRP, BUN, Creatinine
Laboratory Test
Polymorphic markers
New Risk Prediction by Biomarkers
- RANTES
- RAGE
- IL-6
- Adiponectin
- Lp-PLA2
HOMA-IR
Genetic Analysis of CAD susceptible SNPs
- Chr. 2 (1 SNP) - Chr. 5 (1 SNP)
- Chr. 9 (1 SNP) - Chr. 12 (1 SNP)
- Chr. 19 (1 SNP) - LTA (1 SNP)
- AdipoQ (2 SNPs) - RAGE (1 SNP)
- Lp-PLA2 (2 SNPs) - IL-6 (1 SNP)
- RANTES (1 SNP) - PAPPA (1 SNP)
- IL-1β (3 SNPs) - CD14 (1 SNP)
- ABCA1 (1 SNP) - ACE (1 SNP)
- MTHFD1 (1 SNP) - Chr.2 (1 SNP)
- PSRC1 (1 SNP) - MIA3 (1 SNP)
- Chr.10 (1 SNP) – SMAD3 (1 SNP)
- FTO, CDKAL1, HHEX, CDKN2B, IGF2BP2, SLC30A8, KCNJ11
- MTHFR
Interpretation of Risk Prediction
Probability (%)
Myocardial infarction ?
CAD ?

개선된 CAD 발생 위험 예측 모형 구축

CAD 발생 위험 확률 예측을 위한 2-stage model 구축(1,006명)



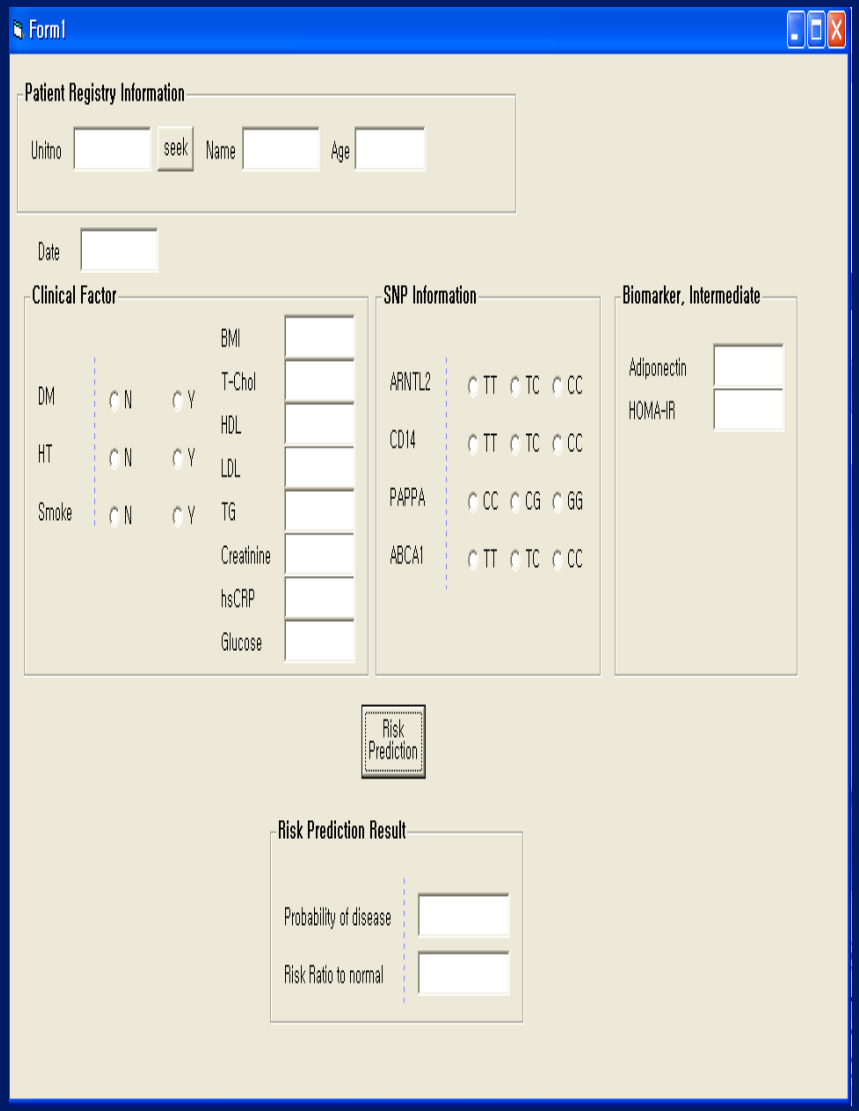
CAD 발생 위험 확률 = $\frac{\exp(-4.0629+0.0622\times GRS)}{1+\exp(-4.0629+0.0622\times GRS)}$

$$\begin{aligned}GRS = 0.8236\times\{&(-0.0189\times ARNTL2_{TT})-(0.0456\times ARNTL2_{TC})-(0.1160\times CD14_{TT})-\\&(0.1213\times CD14_{TC})+(0.0077\times PAPPA_{CC})-(0.0387\times PAPPA_{CG})-\\&(0.0275\times ABCA1_{TT})-(0.0428\times ABCA1_{TC})-(0.0440\times DM)+\\&(0.0143\times HT)-(0.0245\times Smoke)+(0.078\times BMI)-(0.2625\times T\text{-}chol)+\\&(0.0981\times HDL)+(0.3092\times LDL)+(0.1527\times Tg)+(0.2174\times Creatinine)+\\&(0.4784\times Glucose)-(0.0889\times hsCRP)-(0.0007\times Adiponectin)\}\end{aligned}$$

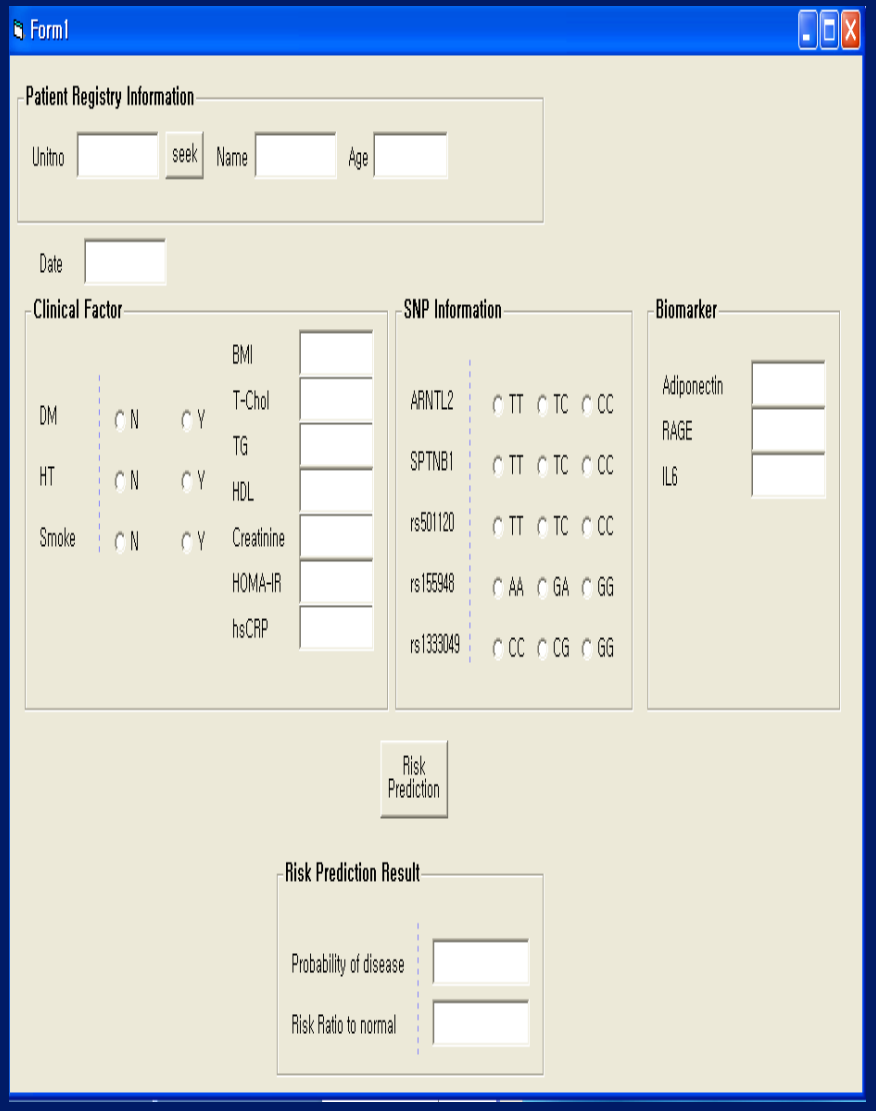
CAD 발생 위험 예측시스템 개발

CAD 발생 위험 예측시스템의 프로그램 실행 화면

Two-stage model을 이용한 CAD 발생 위험 확률 계산 화면



One-stage model을 이용한 CAD 발생 위험 확률 계산 화면



예측모형을 통해 추정된 CAD 발생 위험 확률 (예)

DM	Smoke	BMI	...	hsCRP	ARNTL2	CD14	PAPPA	ABCA1	Probability(%)
No	Yes	normal	...	normal	TC	TC	CG	TC	12.33
No	Yes	normal	...	normal	TC	TT	CG	TC	12.35
No	Yes	normal	...	normal	TC	TC	CG	TT	13.86
					...				
No	Yes	normal	...	normal	TT	CC	CC	CC	50.22
No	Yes	normal	...	normal	CC	CC	GG	CC	50.61
No	Yes	normal	...	normal	CC	CC	CC	CC	53.90
Yes	No	normal	...	normal	TC	TC	CG	TC	12.54
Yes	No	normal	...	normal	TC	TT	CG	TC	12.56
Yes	No	normal	...	normal	TC	TC	CG	TT	14.10
					...				
Yes	No	normal	...	normal	TT	CC	CC	CC	50.71
Yes	No	normal	...	normal	CC	CC	GG	CC	51.10
Yes	No	normal	...	normal	CC	CC	CC	CC	54.39

CAD 발생 위험 예측모형 개발 결과

CAD 예측 실용 모형

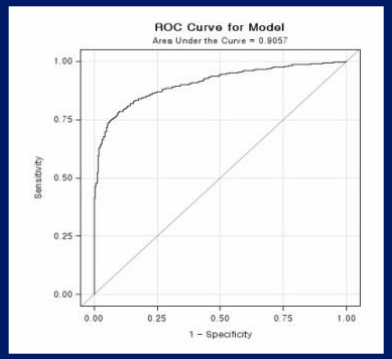
- SNPs

rs11***(ARNTL2), rs12***(SPTNB1),
rs15***(Ch5), rs13***(Ch9)

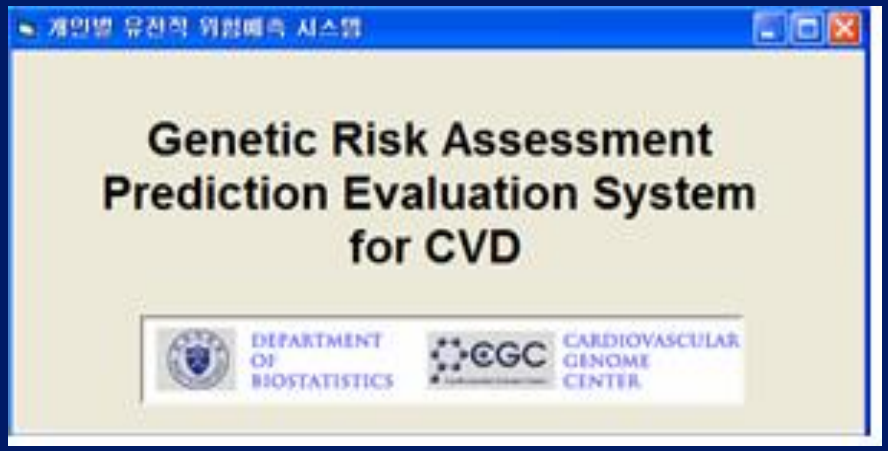
- Conventional risk factor

DM, hypertension, smoke, BMI, Total-cholesterol, Tg, HDL, Creatinine

- 민감도 80.4%, 특이도 84.5%,
  정확도 81.5%, AUC 0.906



타당성 평가
(GenRIC + KCDC 자료)
민감도 100%,
특이도 73.5%,
정확도 86.5%



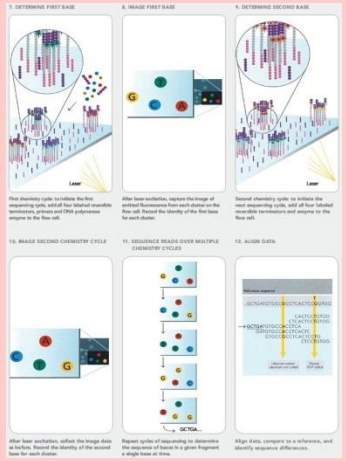
Summary: Identified causative genetic variants for CAD in Korans

Loci	SNP	OR	P	Gene	Function
2p21	rs1687****	1.212	3.01E-06	SPTBN1	Determination of cell shape, arrangement of transmembrane proteins
4q12	rs2124***	1.187	3.27E-05	IGFBP7	Modulation of vascular remodeling
5q	rs1507***	1.285	3.13E-03	*intergenic*	Not known
9p21.3	rs1333***	1.263	3.30E-08	nearby CDKN2A/2B	Regulation of the cell cycle
12q23	rs3782***	1.255	2.13E-05	MYL2	Regulation of myosin ATPase activity in smooth muscle
13q12	rs9508***	1.192	2.34E-05	FLT1	Control of cell proliferation and differentiation

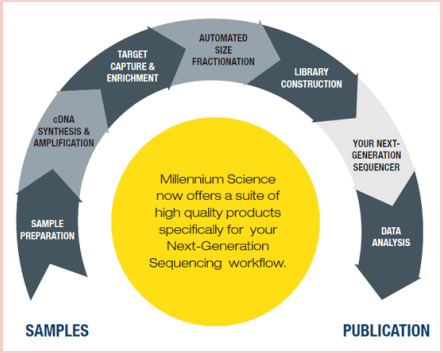
향후 심혈관질환 예측모형 콘텐츠 발굴 전략

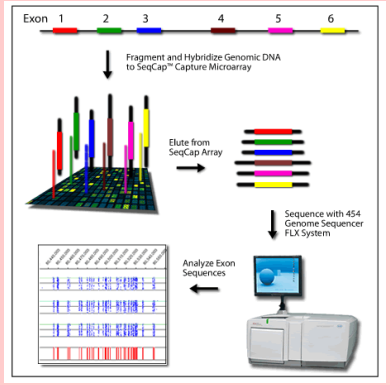
CAD case-control study (CGC)

Candidate gene approach를 통해 유의한 연관성을 나타난
유전자 가운데 GWAS SNP chip에 포함되지 않은 유전자 20종



GenRIC study에서 발굴된
유전자 20종 선발





Target capture NGS analysis

SNP contents validation

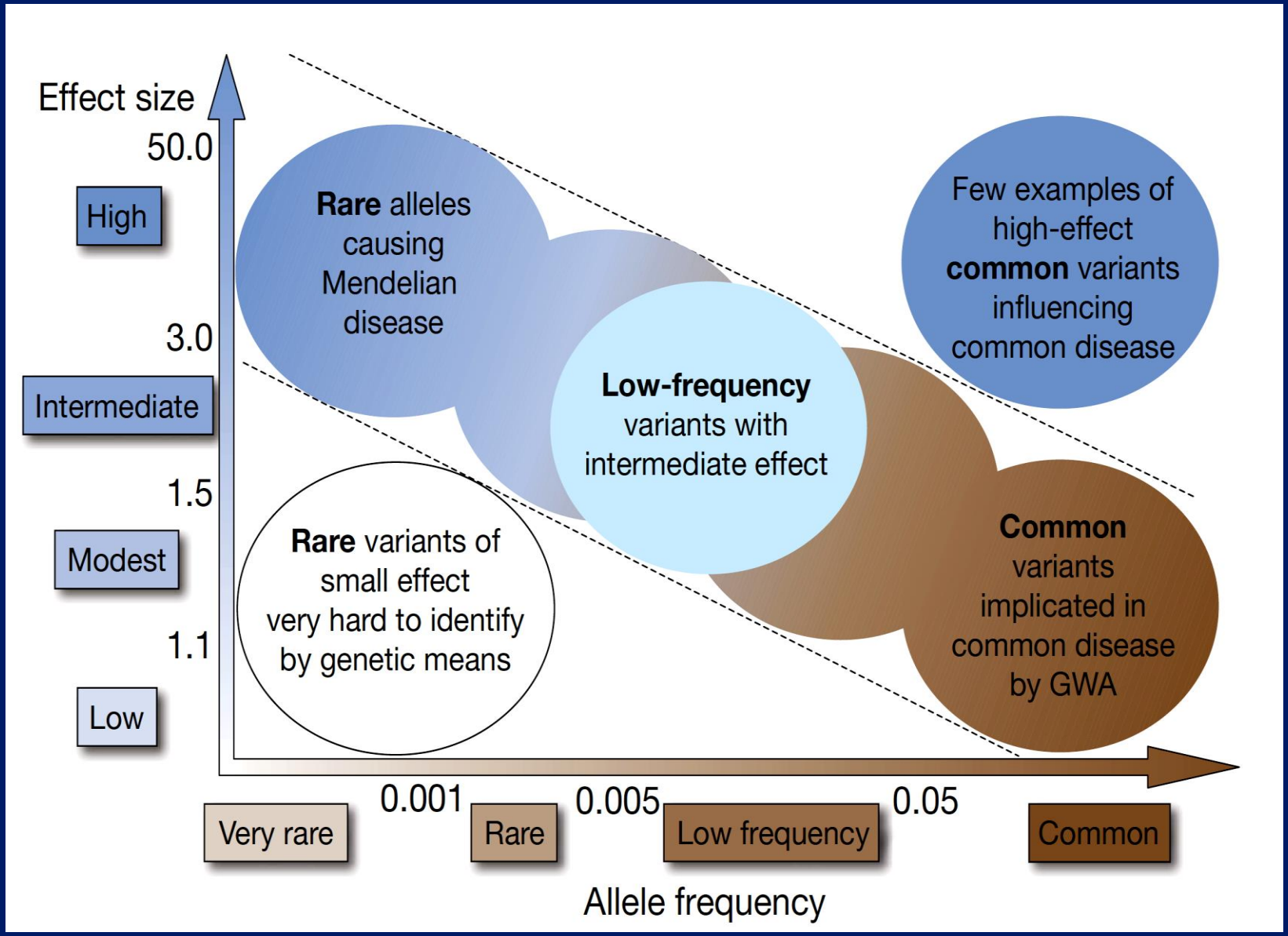
Modest effect of common alleles

Table 3 Prospective studies for 9p21 association with incident CHD risk

Study	Reference	Cohort	Size	Primary outcome*	Follow-up (years)	HR (95% CI) per risk allele	HR (95% CI) risk homozygotes vs reference	Improvement in C-statistic (9p21 plusmodel)	Risk reclassification
Bruneck	62	Population	769	M/D/R/S/P	10	1.35 (1.02 to 1.78)			
Northwick park	97	Population	2742	M/D/R	15		1.57 (1.10 to 2.25)	No	Some
Women's Health Study	98	Population	22 129	M/D/R/S	10.2	1.15 (1.03 to 1.27	1.32 (1.07 to 1.63)	No	No
PMI Study		ACS	733	D/H	9.1		1.08 (0.74 to 1.60)†		
GRACE	104	ACS	3247	M/D	0.5	1.49 (1.03 to 1.98)	–	No	
Peng *et al*	105	ACS	520	M/D/U/F	2.4	NS	NS		
INVEST-GENES	106	CAD + HT	2364	M/D/S	2.8	0.81 (0.66 to 1.00)			
INFORM	106	ACS	557	D/H	3	0.75 (0.59 to 0.95)			
CABG Genomics	107	Post-CABG	845	D	5		1.70 (1.10 to 2.70)†	No	

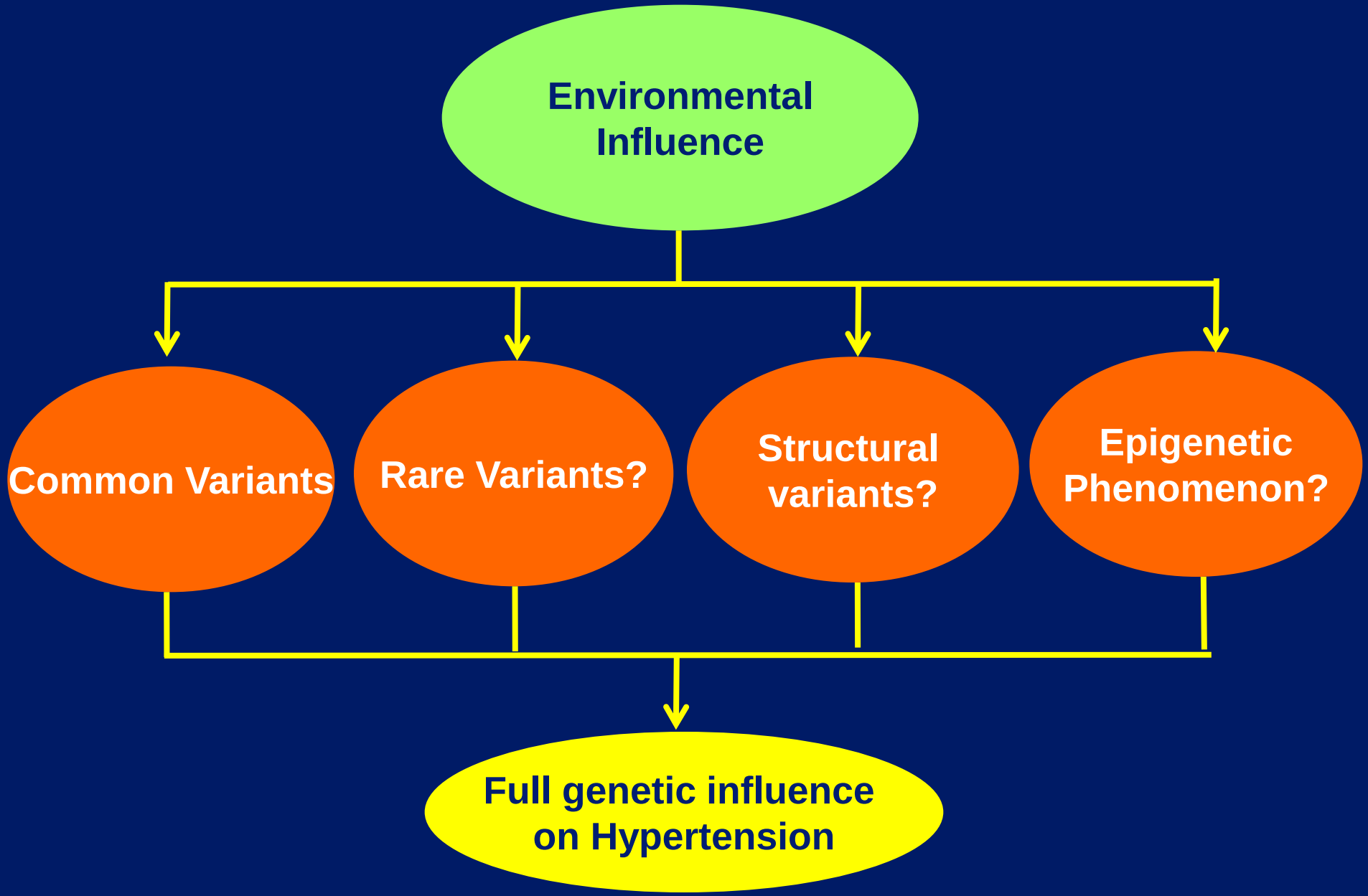
Only 1 study from the ARIC study showed improvement Of CV risk predictability

Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect

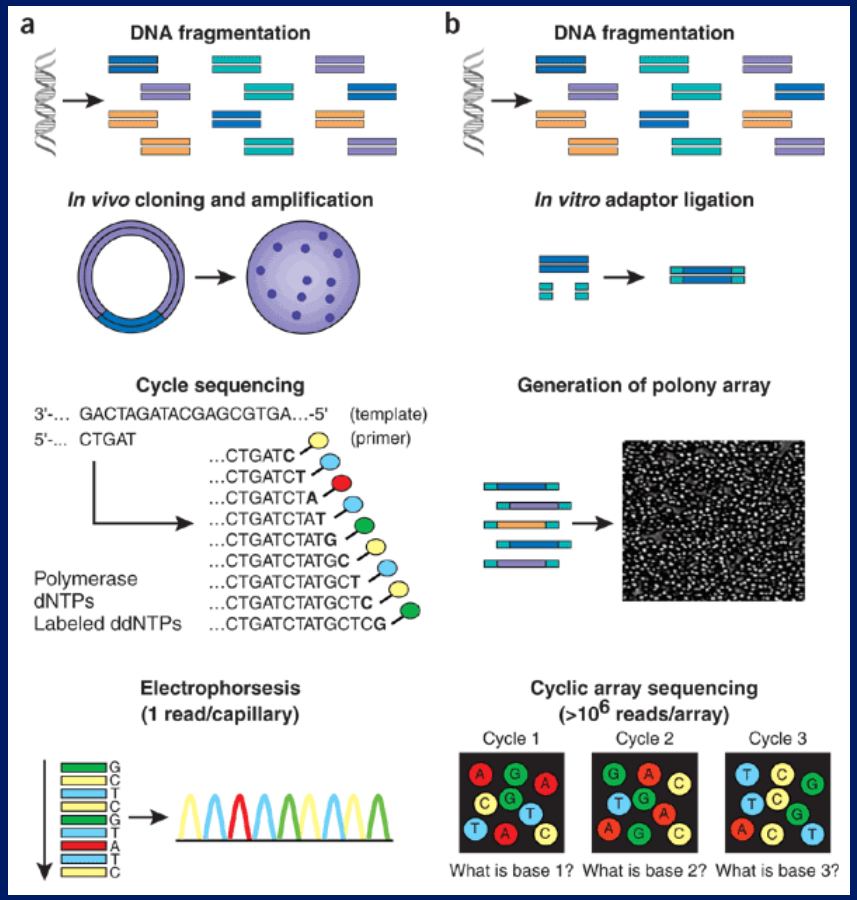


Manolio et al, Nature 2009

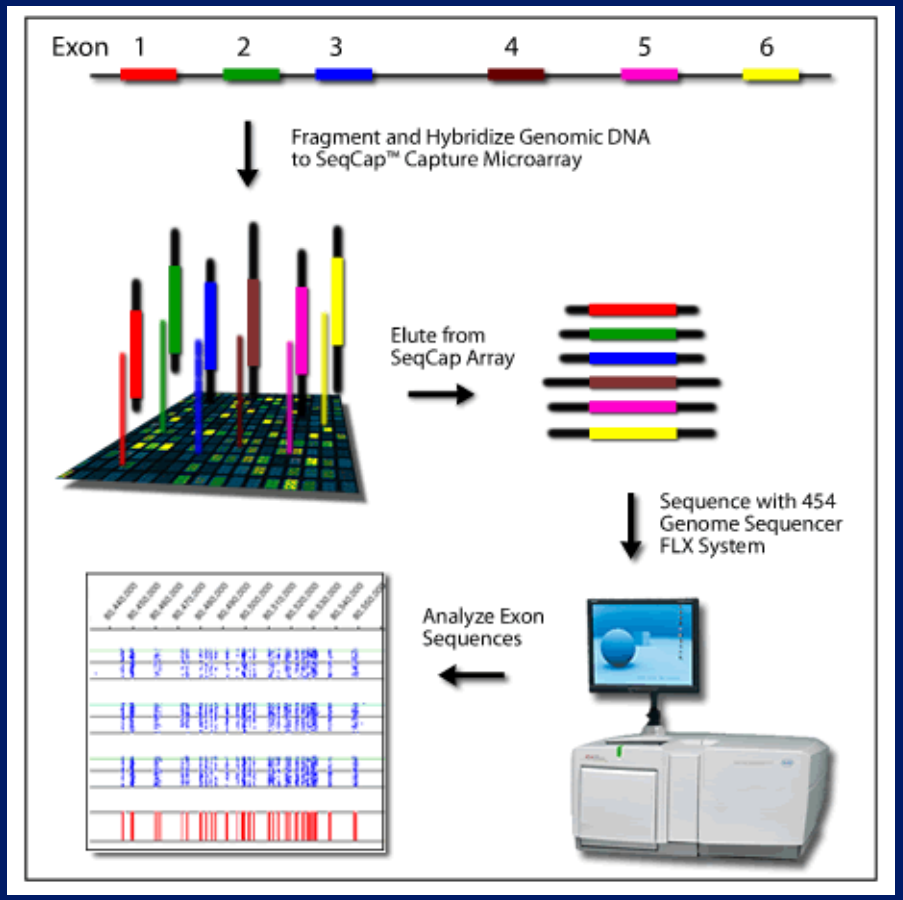
We have only touched the tip of the iceberg



Next Generation Sequencing (NGS)



a) Sanger method (old), b) NGS (new)



Chip-based capturing exome sequencing

Gap between hereditability and effect

- More common variants that need to be discovered
- Gene-gene interaction, gene-environment interaction
- Effect of numerous rare variants(0.5-5% minor allele frequency) with large effects → NGS
- Effect of structural variants in Hypertension pathogenesis → NGS
- Effect of chromosomal structure

  → Epigenomics

Conclusion & Further Study

- Identified several genetic loci that were strongly associated with CAD in worldwide studies by GWAS
- Further studies are needed to survey the associations of the loci with other types of atherosclerotic disease
- At a genetic level, studies should focus on fine mapping of the associated regions using NGS
- Targeted sequencing of CAD-associated multi-gene by NGS is needed for further identification of causative rare variants

Yonsei Cardiovascular Hospital

Yonsei University College of Medicine

Validated SNPs identified in Asian candidate gene studies for CAD

Loci	SNP	OR	P	Gene	Validated population
6p21.2	rs16874954 (V279F)	1.922	0.013	Lp-PLA2	Chinese
		0.80	0.002		Korean
6p21.32	rs2070600 (G82S)	2.303	0.001	RAGE	Chinese
		0.749	0.028		Korean
12q24.12	rs11066001	1.63	$5.0X10^{-11}$	BRAP	Japanese
		1.68	$6.5x10^{-9}$		Korean