Another issue, old but unsolved

Gene and CAD

한림대학교 강동성심병원
심장 내과
이 준 희

CAD has a strong genetic component

- Coronary artery disease – a major cause of death
- CAD – multiple risk factor
  - Obesity, DM, smoking, HT, poor diet, aging population
- but lots of patients have no conventional risk factor
- Large portion of risk for CAD is remained Even after managing known risk factors
- 50% of susceptibility of CAD may be genetic.

Predisposition of CAD is inherited

- The extent of coronary occlusion ∝ parental Hx of MI
  - Anderson et al. Prev med 1979
- Early onset of CAD ∝ greater risk of CAD of relatives
  - In families with CAD onset before 46 yo → hereditability 92 – 100%
  - In families with CAD onset after 46 yo → hereditability 15-30%
  - Rissanen et al. Am J Cardiol. 1979
- Danish twin registry
  - CAD incidence in Monozygotic 44% vs Dizygotic 14%
  - Allen et al. Acta genet stat med. 1967

Genetic basis for CAD

- Premature CAD is genetic
  - MI in 1st degree relatives <55yo → MI risk 7.1x
  - Heritability of early-onset CAD 0.56
    - Lloyd-Jones et al. Lancet. 1999
- Family Hx of CAD – independent risk factor for CAD
  - Framingham study
    - Family Hx of CAD or stroke or peripheral artery disease
    - X2.4 risk in men, x2.2 risk in women
    - Genet et al. Circulation 1992
  - Interheart study
    - x1.45 risk increase
    - Yusuf et al. Lancet 2004

Risk factors for CAD

- Old age
- Hypertension
- Diabetes mellitus
- Dyslipidemia
  - High cholesterol
  - High LDL-C
  - Low HDL-C
- Smoking
- Obesity
- Familial history of premature CAD

From 2007 ESC/ESH guideline for hypertension

CAD is preventable

- CAD is a preventable disease and its elimination is expected before the end of the 21st century

- Elucidation of genetic modifiers is a pre-requisite to genetic screening and comprehensive prevention

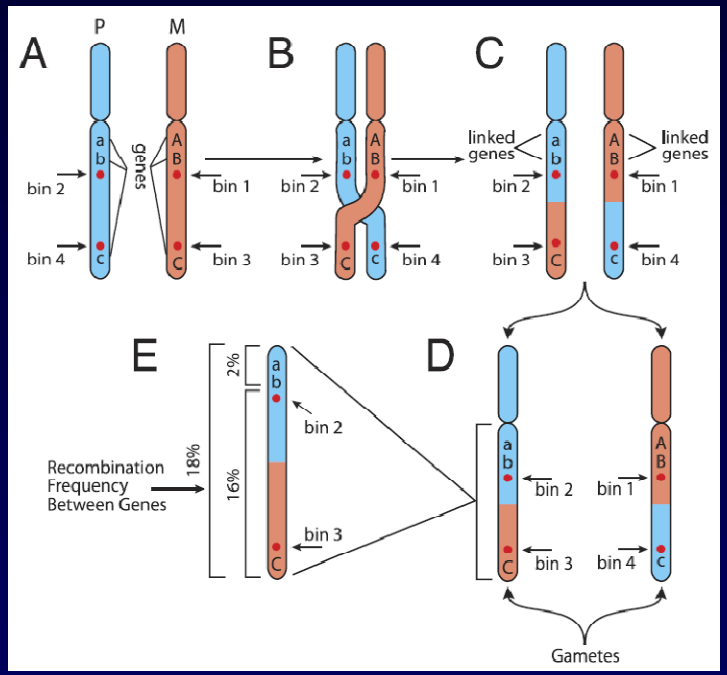
Success in Single gene disorders

- A single gene is necessary and sufficient for disease
- Simple Mendelian traits
  - Autosomal dominant, recessive, X-linked, and mitochondrial
- Rare allelic variants with markedly increase disease risk
- Not great impact on public health

- Familial hypertrophic cardiomyopathy
- Marfan's syndrome
- Long QT syndrome

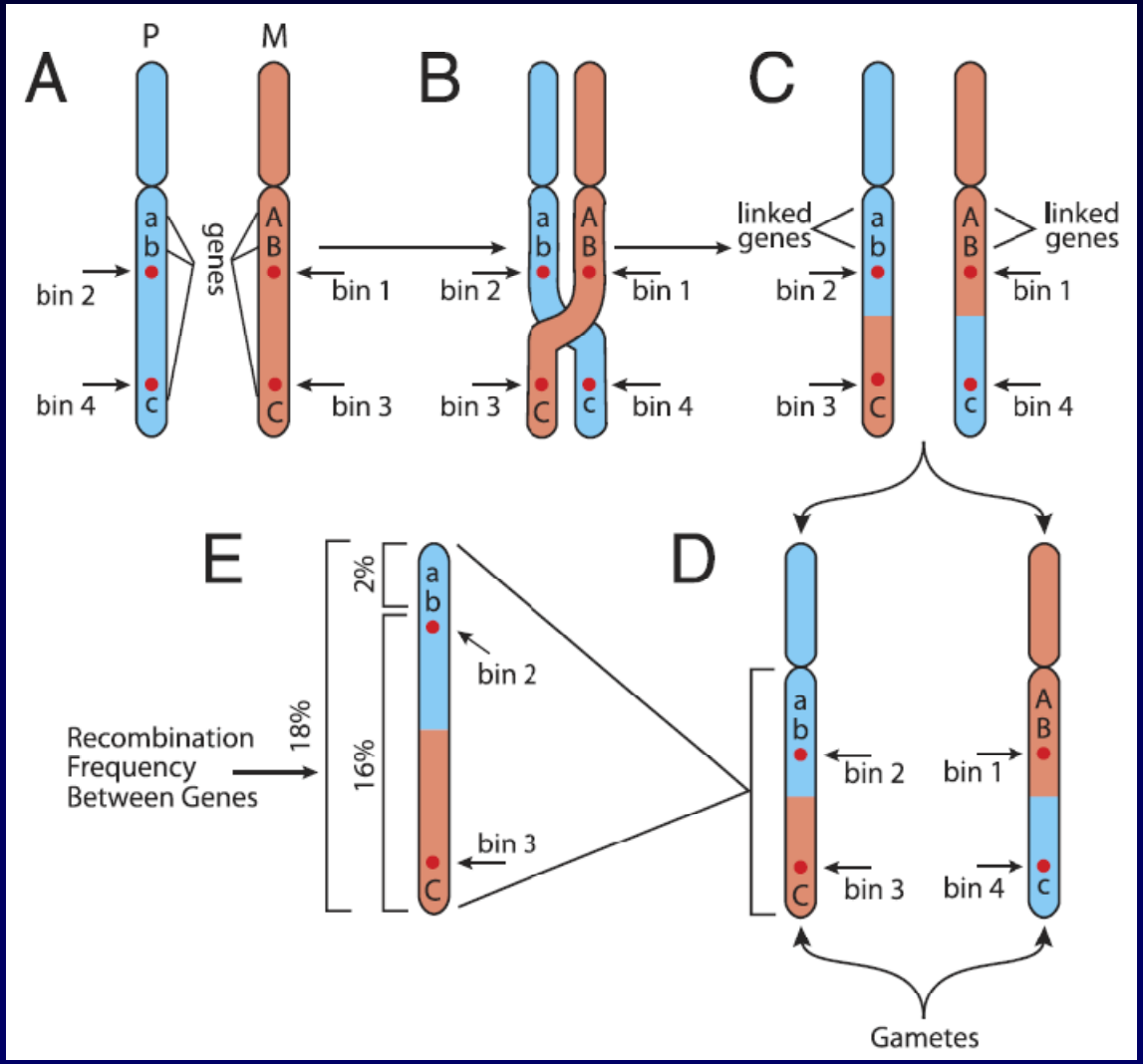
Approach methods

- Single gene disorder
  - DNA from families
  - Pedigrees of 2-3 generation
  - Linkage analysis
    - Linkage disequilibrium
    - 300 DNA markers
      (10M bps interval)
    - Not suitable for detecting
      genes contributing minimal risk



- **CAD → polygenic disorder**

Schematic of genetic linkage



Topol et al. JACC 2007

Difficulties in indentifying genes for CAD

- Polygenic disorder
  - Prevalent genes
  - Only a minor effect to the phenotype (5-10% contribution)
  - Low penetrance

Approach methods

- Single gene disorder
  - Linkage analysis
    - Families
    - Pedigree of 2-3 generation
- Polygenic disorder
  - Case-control association study
    - Unrelated individuals
    - To compare SNP frequency
    - Candidate gene approach (direct method)
    - Genome-wide association study (indirect method)

Candidate gene approach

- Hypothesis
  - Proteins known to be involved in the pathogenesis of atherosclerosis carry mutations or variants that affect their function and ultimately the risk of developing CAD
- Success in only limited number of genes

Gene	Risk-allele frequency (%)	Increase in LDL per risk allele (%)	Effect of risk allele on MI risk	*P*-value for association with MI	Reference
PCSK9	96	+15	OR 1.5	0.0003	24
Apo E	2.8	+14	OR 1.29	0.0001	24
LDLR	11	+6	OR 1.18	0.0001	36
Apo B	33	+5	OR 1.1	0.004	37

Schunkert et al. EHJ 2010

- Reason to failure
  - Restrict a single or few genetic variants with modest impact
  - Small sample size

Gene/polymorphism	Protein encoded	Risk genotype	Patients (number)	Associated with	OR (CI) for ACS	Study
ApoE/ε2, ε3, ε4 alleles	ApoE	ε4 allele	MI (1817)	Lipid metabolism	1.75 (1.19–2.57)	[20]
OLR1/501G>C	OLR1	GC, CC	MI/controls (102/102)	Lipid metabolism	2.89 (1.51–5.53)	[22]
CETP/TaqIB	CETP	No difference	MI/controls (384/384)	Lipid metabolism	RR = 0.95 (0.54–1.66)	[24]
LPL/Gly188Glu, Asp9Asn, Asn291Ser, Ser447Ter	LPL	Gly 188Glu Ser447Ter (protective)	Ischemic heart disease (meta-analysis of 29 studies; 20 903 patients)	Lipid metabolism	4.9 (1.2–20), 0.8 (0.7–1.0)	[23]
eNOS/894G>T, 786T>C, 4a/4b	eNOS	4a4a	ACS/controls (477/537)	Endothelial and vascular function	2.5 (1.1–5.4) for ACS risk, 3.6 (1.2–11.5) for MI risk	[29]
eNOS/894G>T	eNOS	GT,TT	MI/controls (228/519)	Endothelial and vascular function	1.192 (1.131–3.485)	[32]
ACE/I/D	ACE	No difference	MI (4629)	Endothelial and vascular function	1.10 (1.00–1.21)	[33]
AT1R/1166A>C	AT1R	AA/AC in individuals with ACE DD	MI (4629)	Endothelial and vascular function	3.95 with ACE DD genotype	[33]
ATG/rs8007267G>A, rs3783641A>T, rs10483639C>G	GTP-cyclohydrolase I	X haplotype	CAD (347)	Endothelial and vascular function	Not studied	[35]
GPIa/807C>T	GPIa	CT,TT	MI <62 yrs old/MI <49 yrs old (1057/223)	Thrombosis and fibrinolysis	0.57 for patients <62 yrs old, 2.61 for patients <49 yrs old	[36]
GPIa/807C>T	GPIa	CT,TT	MI/controls (219/389)	Thrombosis and fibrinolysis	2.296	[37]
GPIIb/Ser 843	GPIIb	Ser 843	MI/controls (68/346)	Thrombosis and fibrinolysis	1.85 for women under 42 yrs old	[38]
GPIIIa/PI(A2)/PI(A2), PI(A1)/PI(A2), PI(A1)/PI(A1)	GPIIIa	PI(A2)/PI(A2)	Patients, prospective (9149)	Thrombosis and fibrinolysis	RR = 3.6 for patients with MI <40 yrs old	[39]

Tousoulis et al. Trend in molecul med 2008

Thrombospondin 4/Ala387Pro	Thrombospondin	AlaPro, ProPro	Families (men <45 yrs and/or women <50 yrs old MI) (398)	Thrombosis and fibrinolysis	1.89	[40]
PAI-1/4G/4G Haplotype	PAI-1	4G/5G, 4G/4G	70–79 yrs old, MI (177)	Thrombosis and fibrinolysis	No linkage with MI	[43]
MTHFR/677C>T	MTHFR	TT	CHD/controls (11 162/12 758)	Thrombosis and fibrinolysis	1.16 (1.05–1.28)	[44]
IL-6/572G>C, -598C>T	IL-6	CG	ACS/controls (3027/447)	Inflammation	-572 CG genotype was predictive for increased IL-6 levels in patients with a subsequent cardiovascular event, 2.3 (1.1–4.3)	[46]
LTA	LTA	Thr26Asn in coding region, SNP in intron-1	MI/controls (1133/1006)	Inflammation	1.78	[47]
IL-18/SNP rs2043055	IL-18	IL-18 haplotype	856 MI/2688 controls (856/2688)	Inflammation	0.74 (0.64–0.87)	[48]
PAPP-A	PAPP-A	IVS6+95 C allele	MI/controls (170/170)	Inflammation	2.13 (1.12–4.07)	[49]
RANTES/403G>A	RANTES	AG,AA	CAD/controls (2694/530)	Inflammation	1.36 (1.08–1.75)	[50]
CD40LG/3459A>G	CD40L	AA,GG,AG	ACS (2359)	Inflammation	2.50	[51]
TLR4/Asp299Gly	TLR4	GlyGly, AspGly	ACS/controls (183/216)	Inflammation	0.41 (0.18–0.98)	[52]
MMP-9	MMP-9	CT/RQ	MI/controls (1967/3455)	Inflammation	1.25	[53]

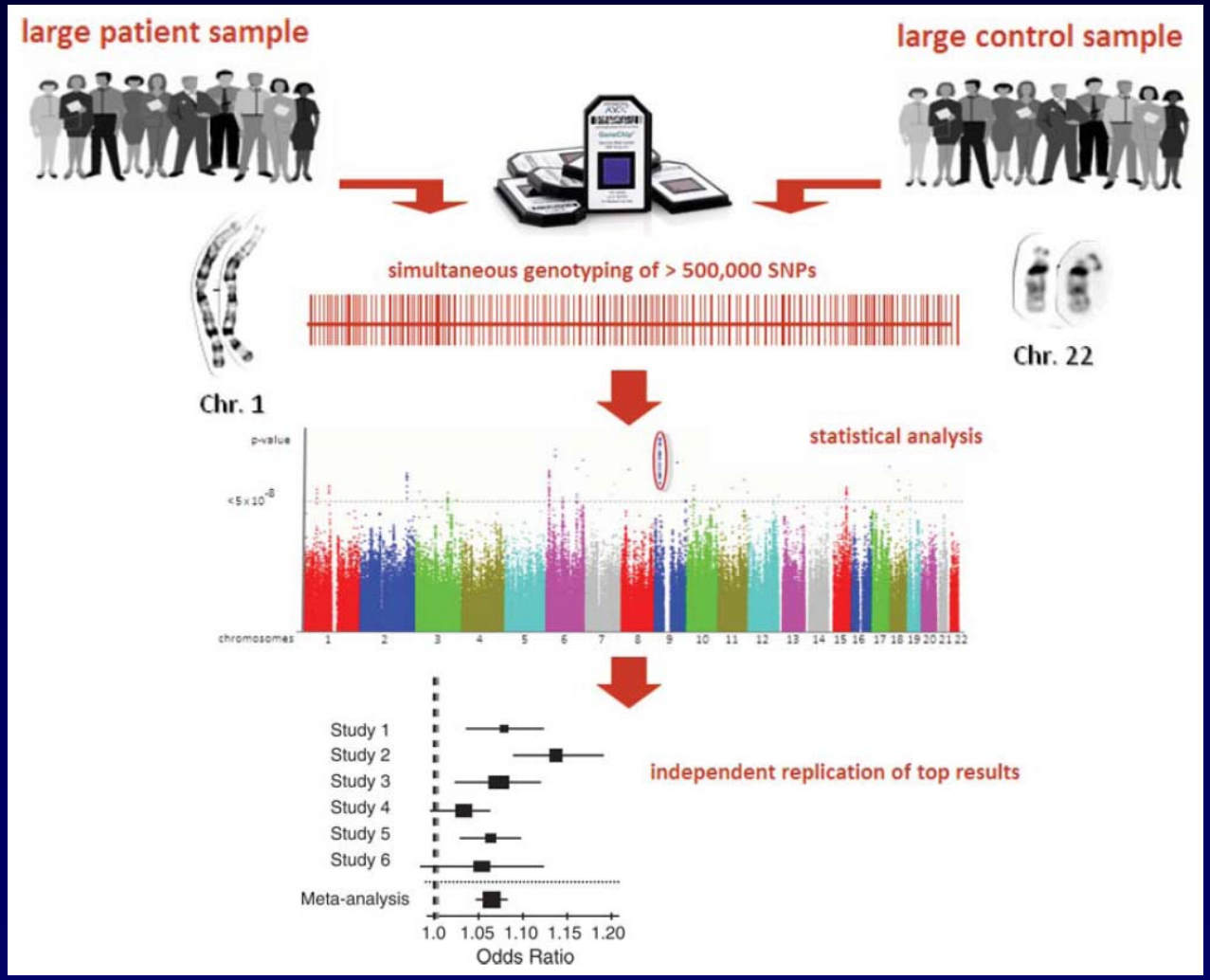
Abbreviations: ACE, angiotensin-converting enzyme; ACS, acute coronary syndromes; ApoE, apolipoprotein E; AT1R, angiotensin II type I receptor; CAD, coronary artery disease; CD40LG, CD40 ligand gene; CETP, cholesterol ester transfer protein; eNOS, endothelial NO synthase; GP, glucoprotein; IL, interleukin; LPL, lipoprotein lipase; LTA, lymphotoxin-A; MI, myocardial infarction; MMP-9, matrix metalloproteinase-9; MTHFR, methylenetetrahydrofolate reductase; OLR1, oxidized LDL receptor 1; PAI-1, plasminogen activator inhibitor 1; PAPP-A, pregnancy-associated plasma protein-A; RANTES, regulated upon activation, normal T-cell expressed and secreted; TLR-4, Toll-like receptor 4.

Tousoulis et al. Trend in molecul med 2008

New strategy is needed

- Genome-wide association study (GWAS)
  - Preferred method
  - No prejudice, no presumption, all inclusive
  - SNP as markers
    - 1 SNP per 1000bps (3,000,000 SNPs in a genome)

Principle of GWAS



Schunkert et al. EHJ 2010

Difficulties in indentifying genes for CAD

- Polygenic disorder
  - Prevalent genes but of low penetrance
  - Only a minor effect to the phenotype (5-10% contribution)
- Genome-wide association study
  - Hundreds of thousands of markers
  - Thousands of unrelated individuals
  - => lots of cost, money, effort

What makes GWAS possible?

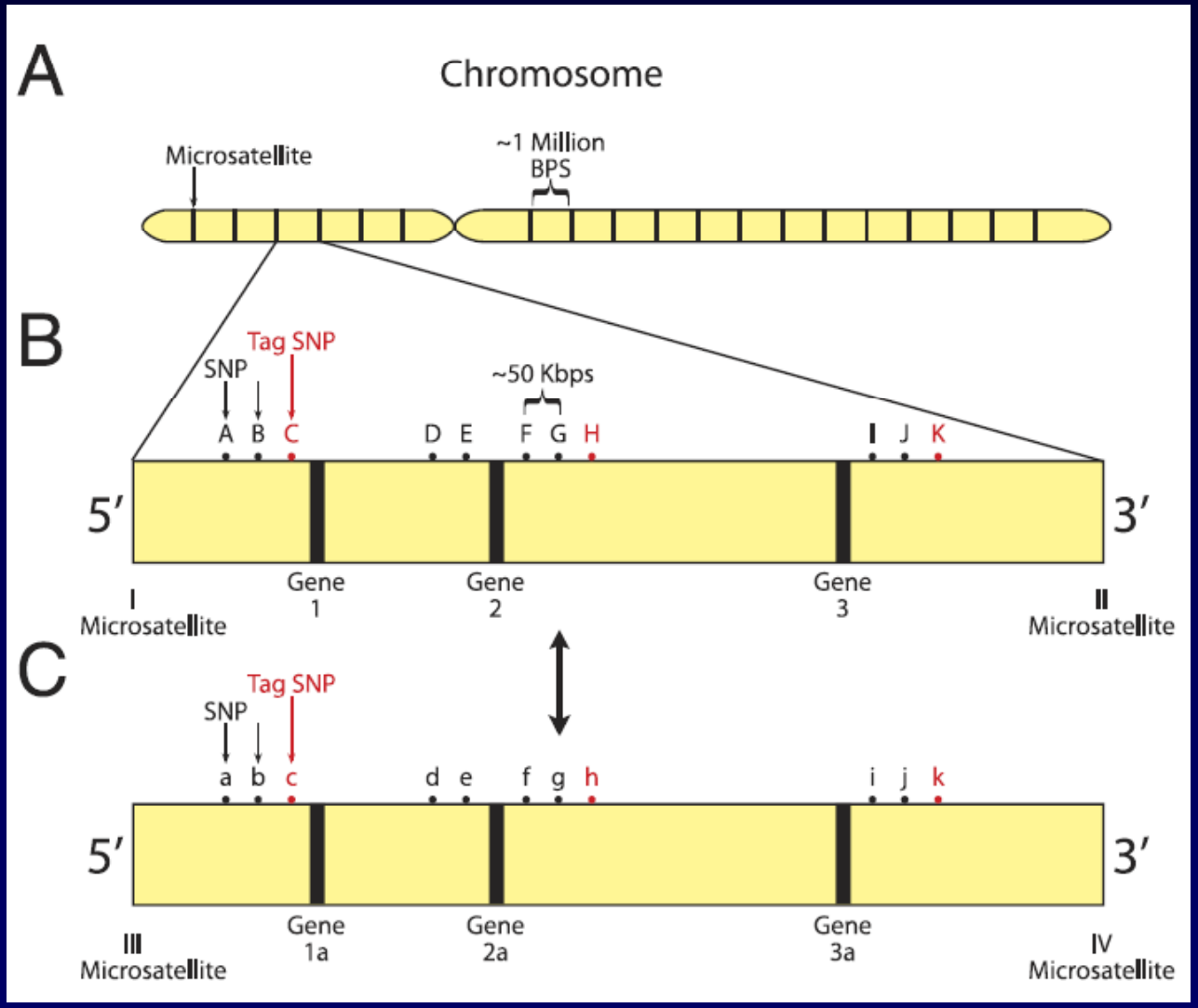
- Human genome project (2003)
  - sequencing of 3,000,000,000 bps of human genome
  - Cataloging of common SNPs by SNPs consortium
  - Establishment of the relationship btw adjacent SNPs by HapMap Consortium
- DNA chip (before 2000)
  - Can analyze millions of SNPs at a time

SNPs (single nucleotide polymorphisms)

- Human genome = 3,000,000,000 bps
- Variation in human genome is 0.1% → most of them are SNPs
- SNP – 1 per 1000bps => 3,000,000 SNPs in a genome

- Majority of human variation and susceptibility to disease is due to SNPs
- Each SNP can explain only 5-10% of susceptibility to disease

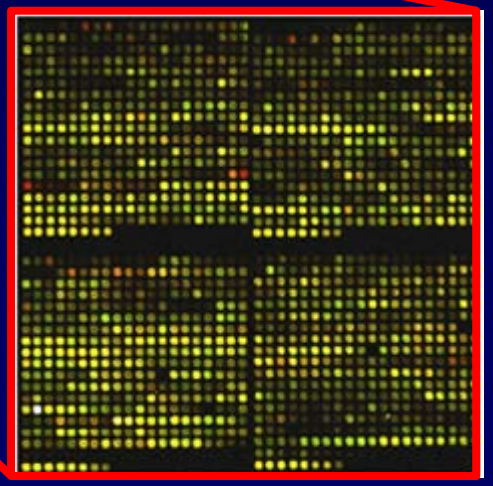
Genomic markers



Microsatellite 400
SNP 3,000,000
Tag SNP 250,000 ~ 500,000

DNA chip

- Affymetrix Genechip
- microarray
- 250,000 SNP on a chip



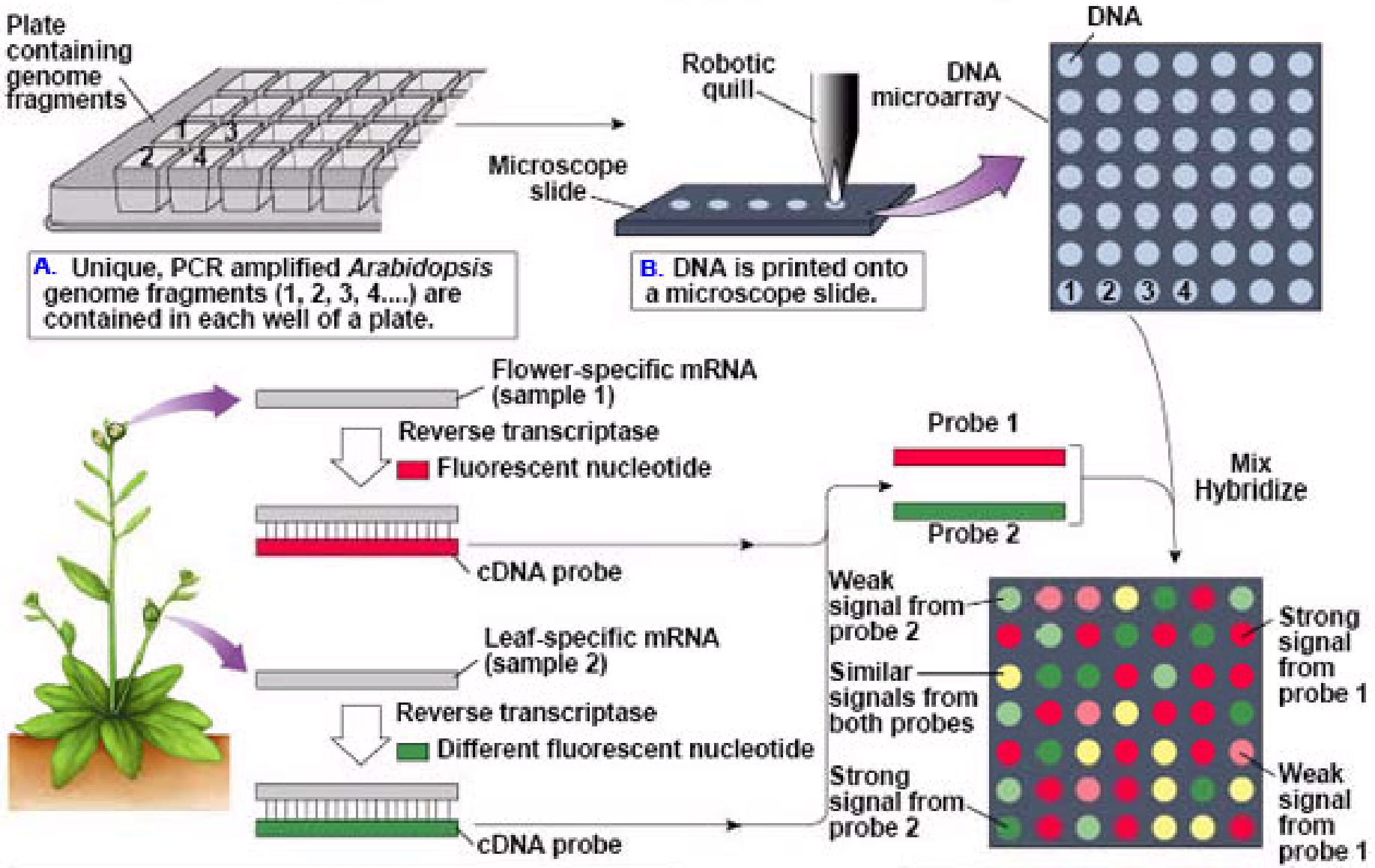
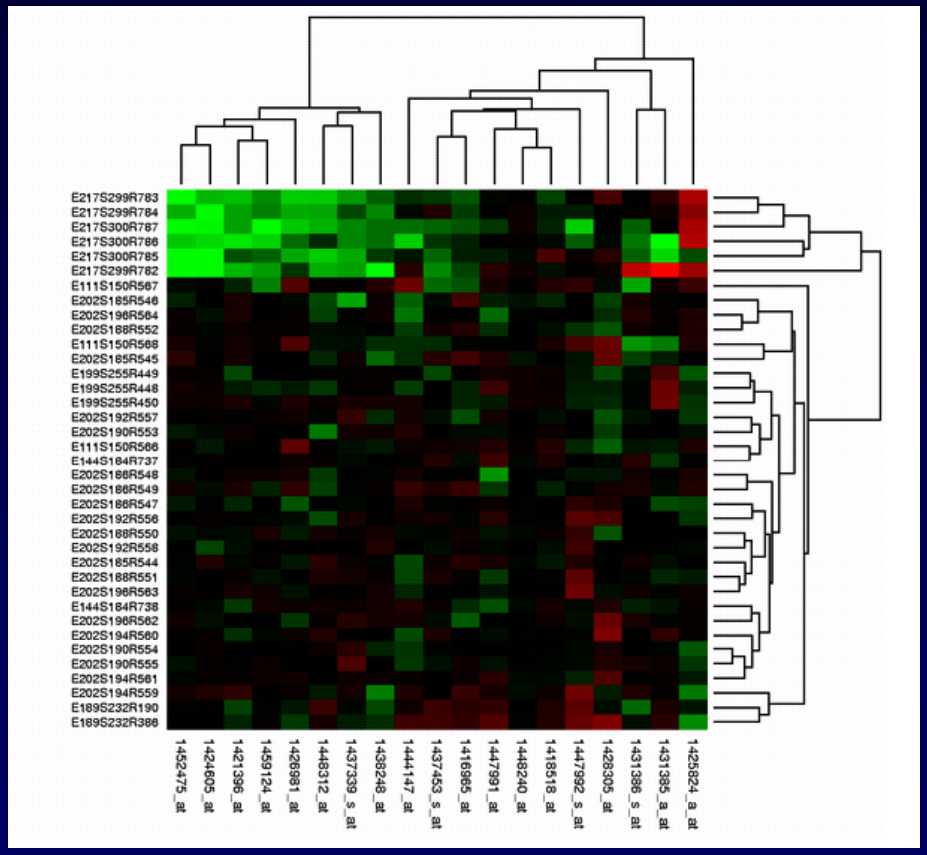


Plate containing genome fragments
1
2
3
4
Robotic quill
Microscope slide
DNA microarray
DNA
1 2 3 4
A. Unique, PCR amplified *Arabidopsis* genome fragments (1, 2, 3, 4....) are contained in each well of a plate.
B. DNA is printed onto a microscope slide.
Flower-specific mRNA (sample 1)
Reverse transcriptase
Fluorescent nucleotide
cDNA probe
Leaf-specific mRNA (sample 2)
Reverse transcriptase
Different fluorescent nucleotide
cDNA probe
Probe 1
Probe 2
Mix Hybridize
Weak signal from probe 2
Similar signals from both probes
Strong signal from probe 2
Strong signal from probe 1
Weak signal from probe 1
C. Samples of mRNA are obtained, for instance from two different tissues. Probes for each sample are prepared using a different fluorescent nucleotide for each sample.
D. The two probes are mixed and hybridized with the microarray. Fluorescent signals on the microarray are analyzed.


E217S299R783
E217S299R784
E217S300R787
E217S300R786
E217S300R785
E217S299R782
E111S150R567
E202S185R546
E202S196R564
E202S188R552
E111S150R568
E202S185R545
E199S255R449
E199S255R448
E199S255R450
E202S192R557
E202S190R553
E111S150R566
E144S184R737
E202S186R548
E202S186R549
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E202S188R551
E202S196R563
E144S184R738
E202S196R562
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E202S194R559
E189S232R190
E189S232R386
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1421396_at
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1426981_at
1448312_at
1437339_s_at
1438248_at
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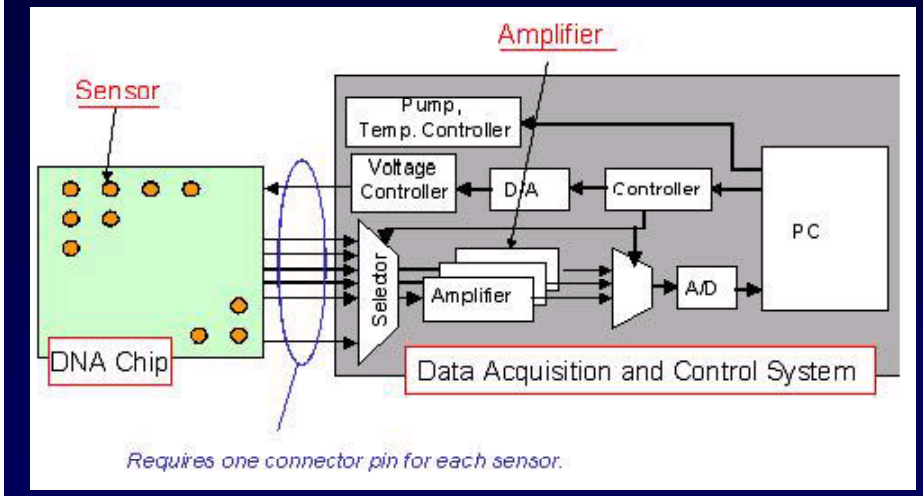

Amplifier
Sensor
Pump,
Temp. Controller
Voltage
Controller
D/A
Controller
PC
Seletor
Amplifier
A/D
DNA Chip
Data Acquisition and Control System
Requires one connector pin for each sensor.

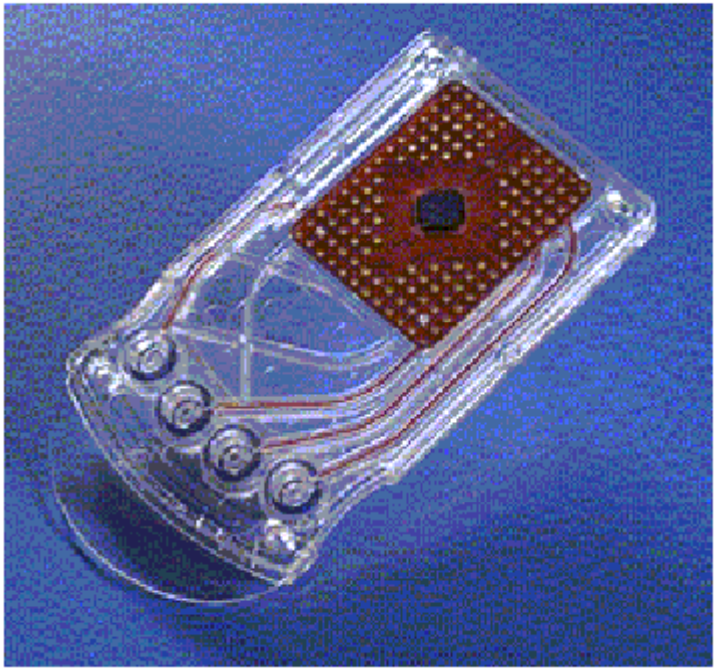


그림 2-4. Nanogen의 DNA chip

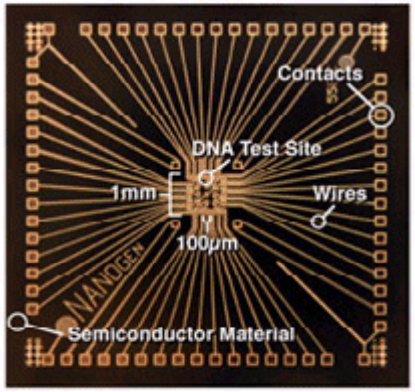


그림 2-6. DNA chip

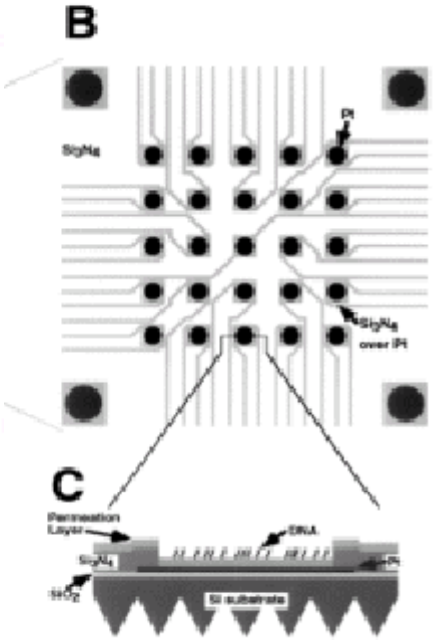


그림 2-5. Microelectrodes

Electronic addressing/ hybridization

Design of GWAS

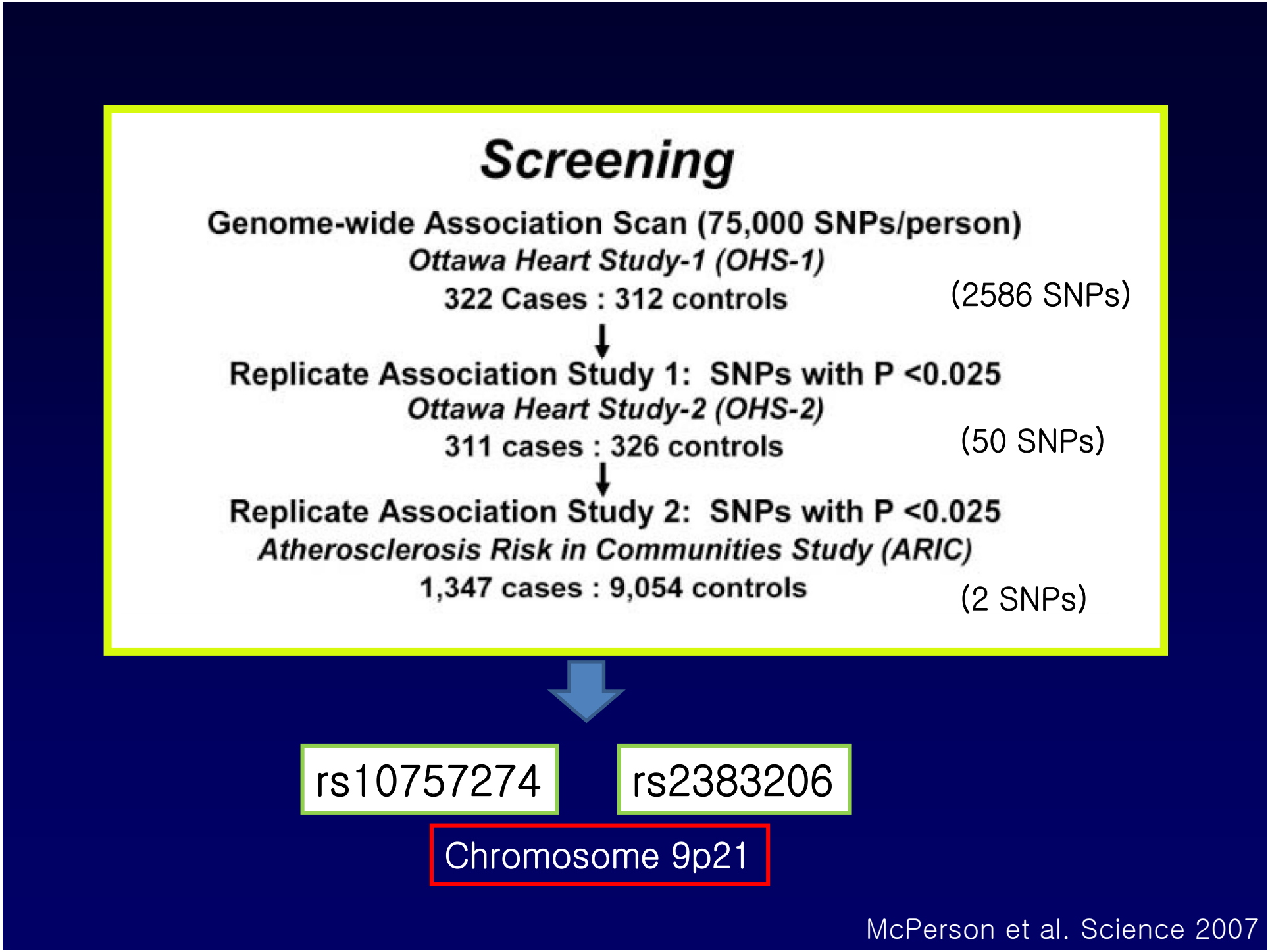
- Calculation of sample size
  - To find gene for >30% increase risk
  - To detect alleles with frequency > 5%
  - Size difference >0.2
  - 90% power

  - Initial population 2000 (1000 affected, 1000 control) for 500K marker set with $p < 0.001$
  - Replication is absolutely essential with an independent large population to rule out false positive
  - Second population 12,000 (8000 affected, 4000 control) for strong association with $p < 0.000001$

Ottawa heart genomics study

- Planned in 2004
- Total sample size 14000
- Criteria for premature CAD
  - Male <55yo or female <65yo
  - Absence of DM (untreated HbA1C <6%)
  - LDL <5.0mmol/L
  - BP < 140/90mmHg
  - CAD confirmed by CAG or CTA
- Criteria for control subject
  - Asymptomatic men >65yo and women >70yo
  - Matched for sex, plasma lipid, HbA1C, BP
  - CTA to exclude coronary atherosclerosis

McPerson et al. Science 2007


Screening
Genome-wide Association Scan (75,000 SNPs/person)
Ottawa Heart Study-1 (OHS-1)
322 Cases : 312 controls
(2586 SNPs)
Replicate Association Study 1: SNPs with P <0.025
Ottawa Heart Study-2 (OHS-2)
311 cases : 326 controls
(50 SNPs)
Replicate Association Study 2: SNPs with P <0.025
Atherosclerosis Risk in Communities Study (ARIC)
1,347 cases : 9,054 controls
(2 SNPs)
rs10757274
rs2383206
Chromosome 9p21
McPerson et al. Science 2007

rs10757274 rs2383206

Chromosome 9p21

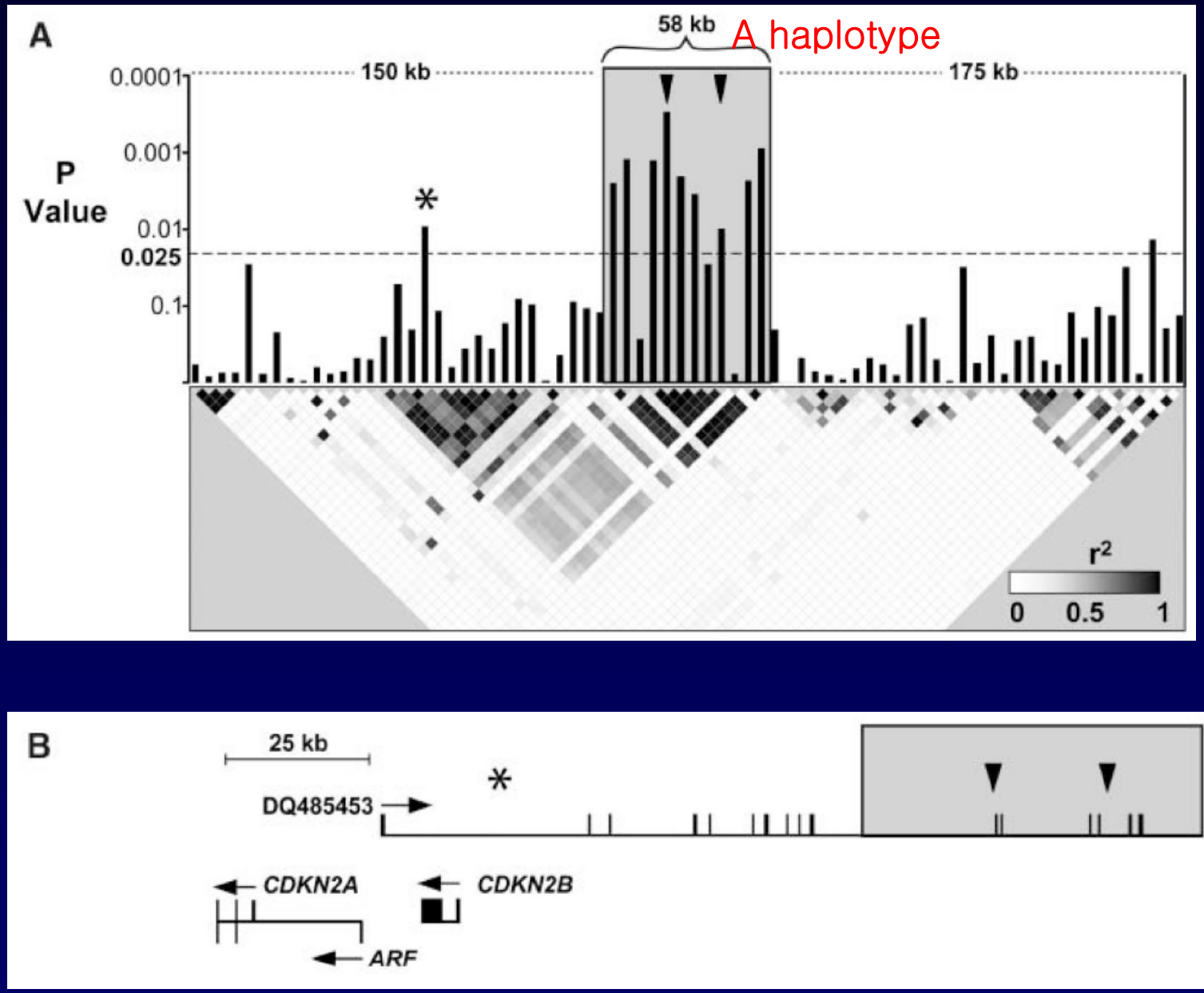
Validation

Copenhagen City Heart Study (CCHS)	*Dallas Heart Study (DHS)*	*Ottawa Heart Study-3 (OHS-3)*
1,525 cases	154 cases	647 cases
9,053 controls	527controls	847 controls

McPerson et al. Science 2007

		CCHS			
		Number of events			
	n (%)	Observed	Expected	Incidence	Hazard ratio
	rs10757274				
AA	3145 (30)	393[†]	473	61 (55–68)	1
AG	5335 (50)	792	755	73 (68–79)	1.26 (1.12–1.42)
GG	2098 (20)	340	296	80 (72–89)	1.38 (1.19–1.60)
	rs2383206				
AA	2,861 (27)	372[§]	425	64 (58–71)	1
AG	5,365 (51)	782	772	72 (67–77)	1.16 (1.02–1.31)
GG	2,352 (22)	371	327	78 (71–87)	1.29 (1.12–1.50)

McPerson et al. Science 2007

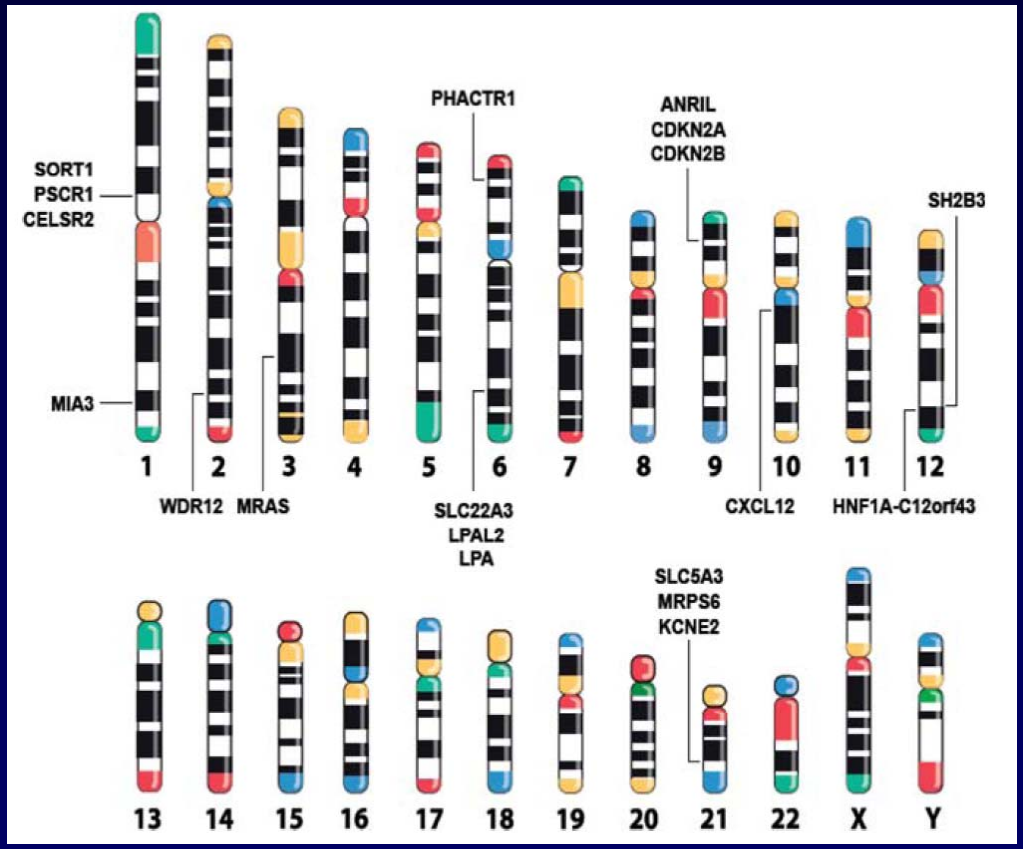


McPerson et al. Science 2007

9p21 is the 1st genetic risk variant

- 9p21 genetic risk variant is extremely common
- 75% of caucasians have 1-2 risk alleles
  - 50% heterozygotes (15-20% increased risk)
  - 25% homozygotes (30-40% increased risk)
- Confirmed in several independent groups
  - Iceland, British, German, central Europe
  - Korean, Japanese, Chinese
  - African-American : not confirmed
- Risk factor for CAD, AAA, intracranial aneurysm, stroke
- Independent of conventional risk factors (DM, HT, lipid, obesity..)

Genetic loci for CAD/MI



Schunkert et al. EHJ 2010

Genetic loci for CAD/MI

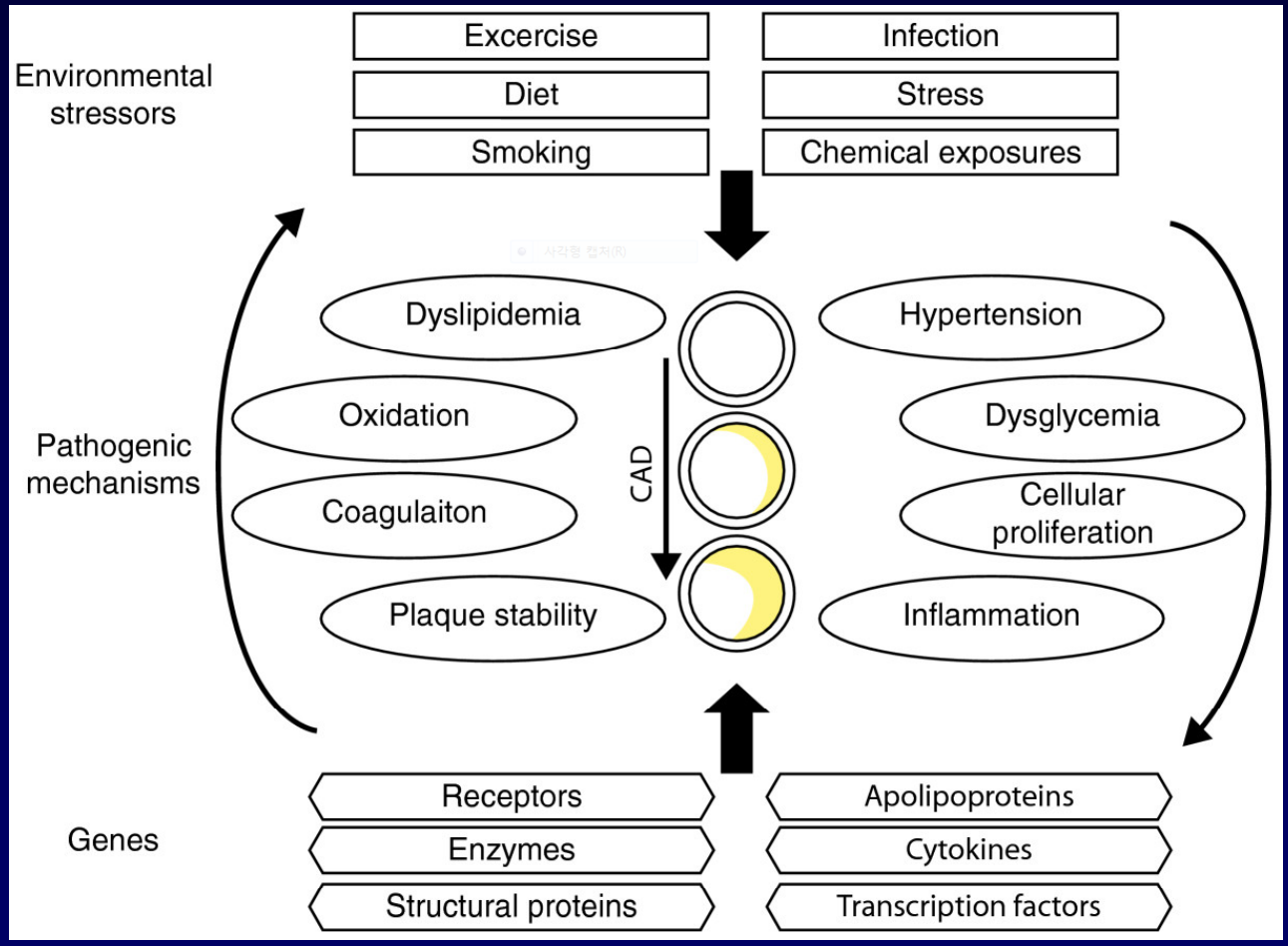
Band	SNP	Risk-allele frequency in Europeans (%)	OR (95% CI)	*P*-value	Genes	Underlying mechanism	References
1p13.3	rs599839	77	1.13 (1.08–1.19)	1.1×10^{-14}	*PSCR1, CELSR2, SORT1, MYBPHL*	LDL	11,38
1q41	rs3008621	72	1.10 (1.04–1.17)	1.4×10^{-9}	*MIA3*	Unknown	11,16,38
2q33	rs6725887	14	1.17 (1.11–1.23)	1×10^{-8}	*WDR12, ALSC2R13*	Unknown	15
3q22.3	rs9818870	15	1.15 (1.11–1.19)	7.4×10^{-13}	*MRAS*	Unknown	15
6p24	rs12526453	65	1.13 (1.08–1.17)	1×10^{-9}	*PHACTR1*	Unknown	16
6q26-27	rs2048327 rs3127599 rs7767084 rs10755578	18	1.20 (1.13–1.28)	1.2×10^{-9}	*SLC22A3, LPAL2, LPA*	Lp(a)	18
9p21.3	rs1333049	52	1.20 (1.16–1.25)	2.8×10^{-21}	*MTAP, CDKN2A, CDKN2B, ANRIL*	Unknown	11–13,39
10q11	rs501120	84	1.11 (1.05–1.18)	9.5×10^{-8}	*SDF1*	Unknown	11,38
12q24	rs11065987	34	1.14 (1.10–1.19)	5.2×10^{-11}	*SH2B3*	Unknown	17,40
12q24.3	rs2259816	36	1.08 (1.05–1.11)	4.8×10^{-7}	*HNF1A, C12orf43*	Unknown	15
21q22	rs9982601	13	1.19 (1.14–1.27)	6×10^{-11}	*SLC5A3, MRPS6, KCNE2*	Unknown	16

Schunkert et al. EHJ 2010

Presumed new mechanisms of CAD

- 9p21
  - No known protein coding region
  - Increase expression of ANRIL (large antisense non-coding RNA gene), p15, p16, ARF in patients in CAD, stroke, AAA
  - Risk allele (+) → enhancer activity alteration → ANRIL expression → modulating expression of genes controlling cellular proliferation pathway
- 3q22
  - MRAS (muscle RAS oncogene homolog)
  - Suggest a plausible role for MRAS in adhesion signaling

Gene-environment interaction



Summary and conclusion

- Modern genetics open up an entirely new era
- Pathogenesis by genetic RF is largely independent of that by traditional RF
  - New pathogenesis of CAD can be reveals though GWAS
- Genetic RF may require a specific environment to come into effect
  - A better knowledge of these interaction will be vital to gain the greatest benefit from this genetic information
- More comprehensive risk assessment and prevention will be possible in the future