Nutrigenomics and Cardiovascular Disease

Brian J Bennett, PhD

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University of North Carolina, Chapel Hill

Outline

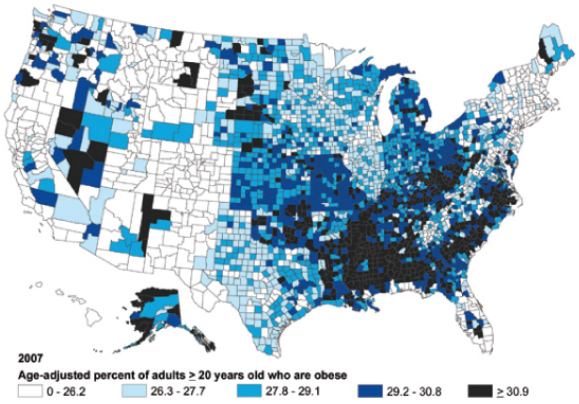
- Introduction
- Candidate genes and nutri-genetic studies
- GWAS
- GWAS candidates and nutri-genetic
- Mouse studies

Why CVD?

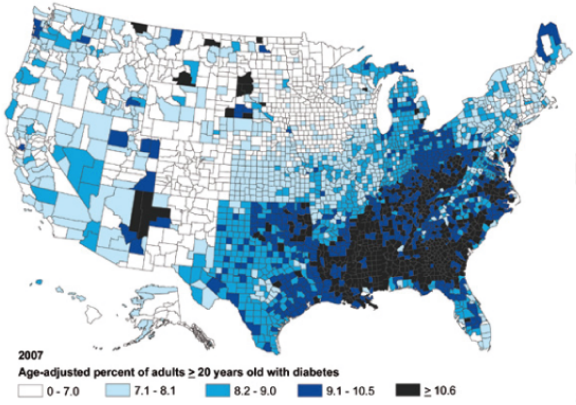
- CVD is the leading cause of death in the US
- In 2010, total costs of CVD were estimated to be $444 billion.
- Treatment accounts for about $1 of every $6 spent on health care in the US.

National Epidemic of Metabolic Disease

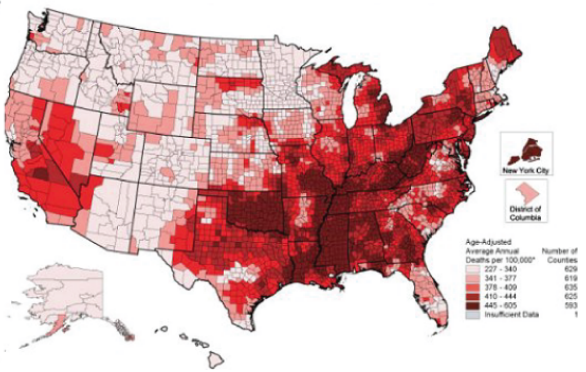
Obesity



Diabetes

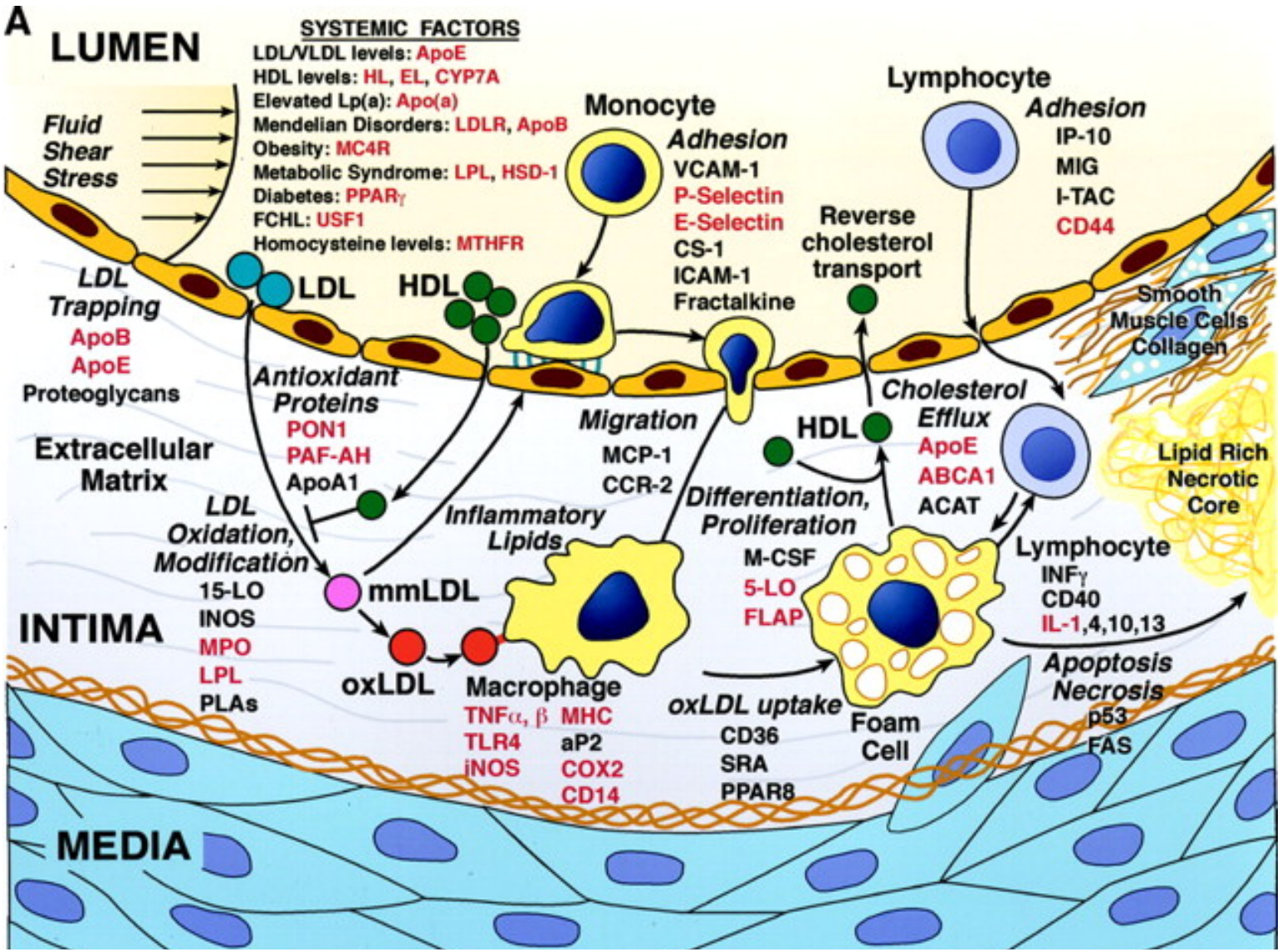


Heart Disease



1. As geneticist we know there are genetic factors
2. Clearly there are environmental factors
3. As nutrition scientist: diet is an important environmental factor

Atherosclerosis is a complex disease involving multiple genes, cell types and metabolic process at the vessel wall



Aldons J. Lusis et al. Circulation. 2004;110:1868-1873

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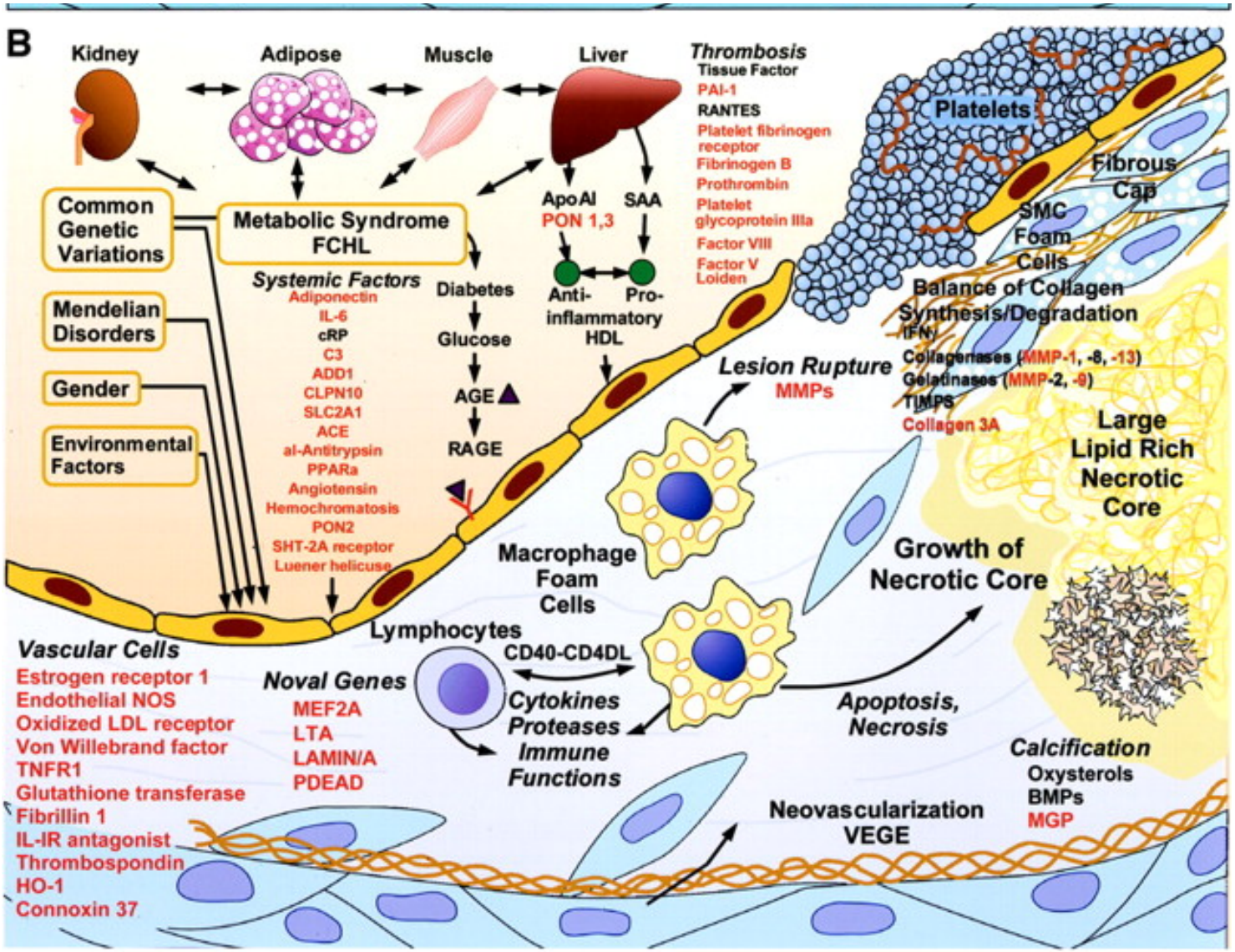
Atherosclerosis is a complex disease involving multiple genes, cell types and metabolic process at the vessel wall



Aldons J. Lusis et al. Circulation. 2004;110:1868-1873

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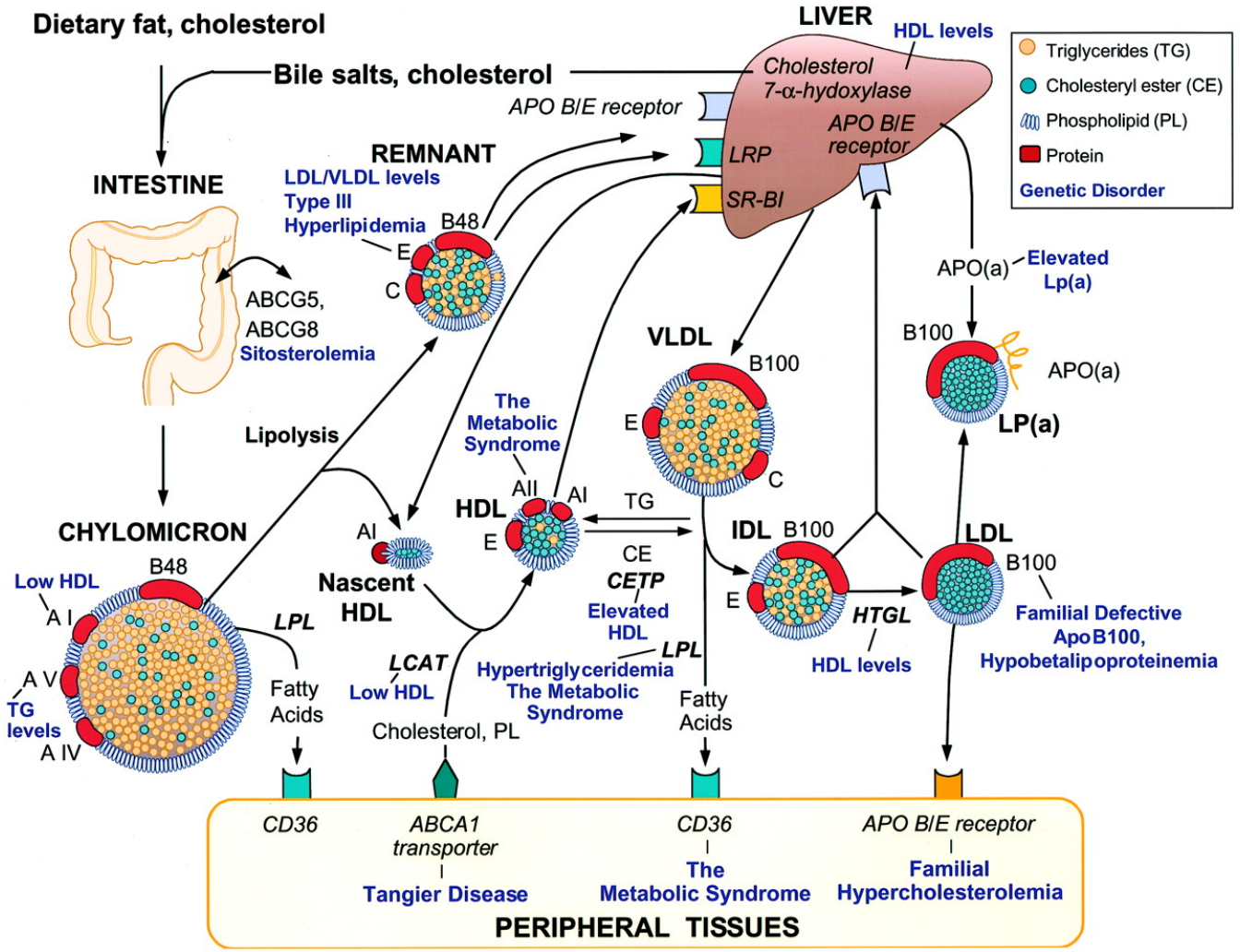
Candidate genes in lipoprotein metabolism and atherosclerosis



Aldons J. Lusis et al. Circulation. 2004;110:1868-1873

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Why PUFA's?

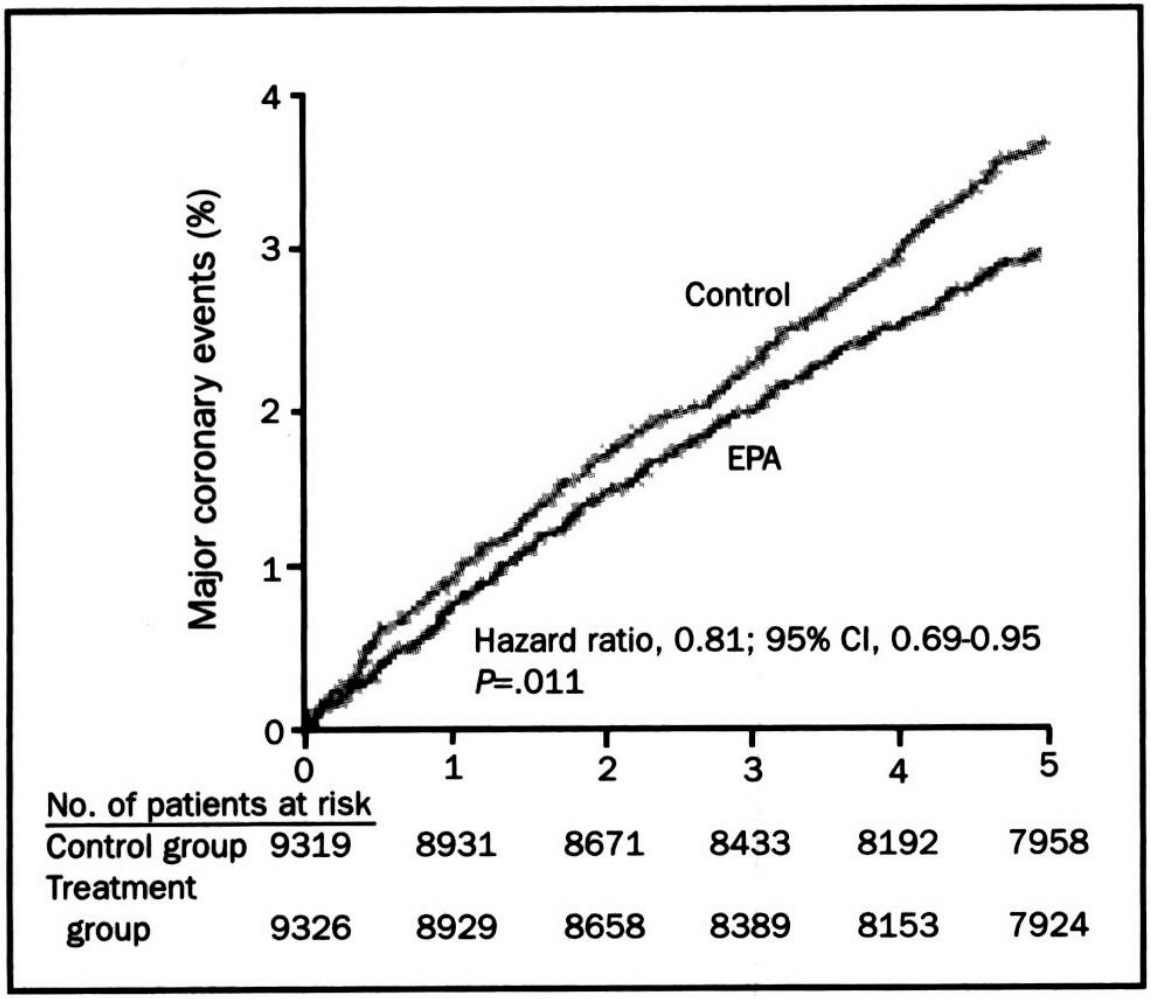


FIGURE 2. Eicosapentaenoic acid (EPA) (1.8 g/d) reduced the incidence of major adverse coronary events in the Japan EPA Lipid Intervention Study (JELIS) by 19%. CI = confidence interval. From *The Lancet*,[8] with permission from Elsevier.

Potential cardio-protective mechanisms of PUFA's

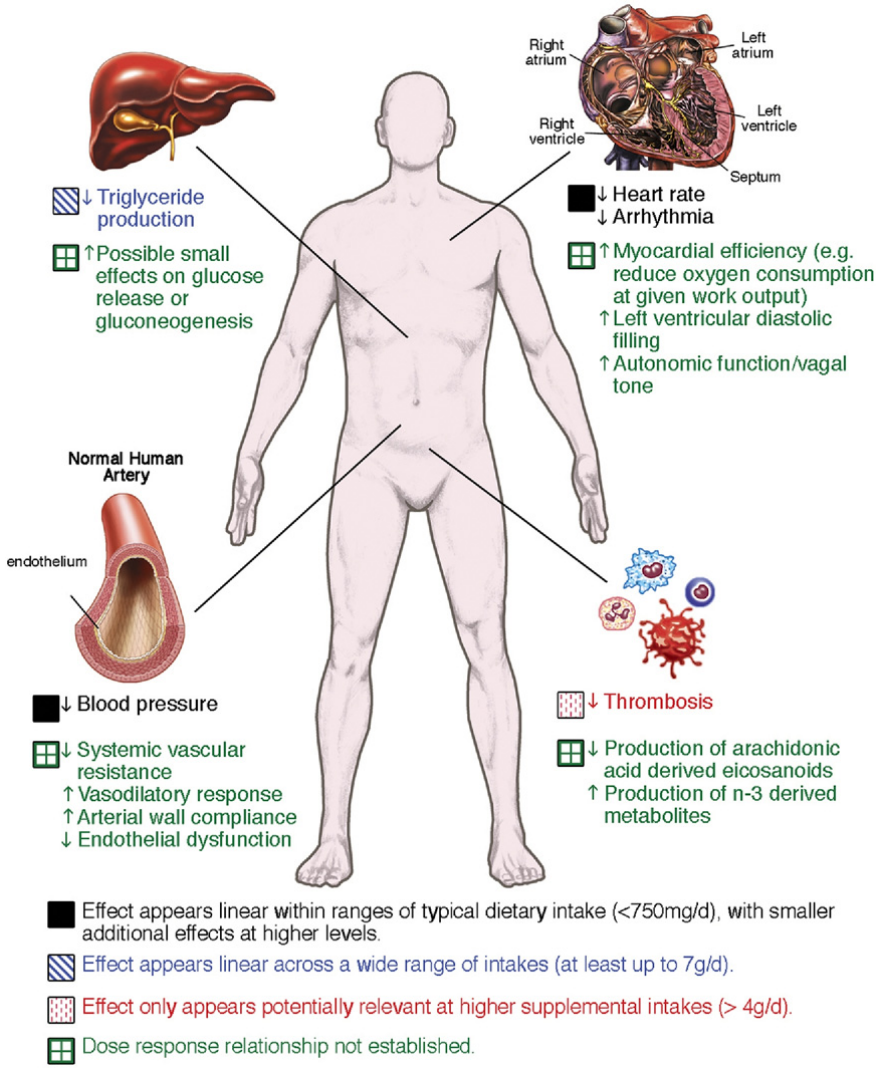
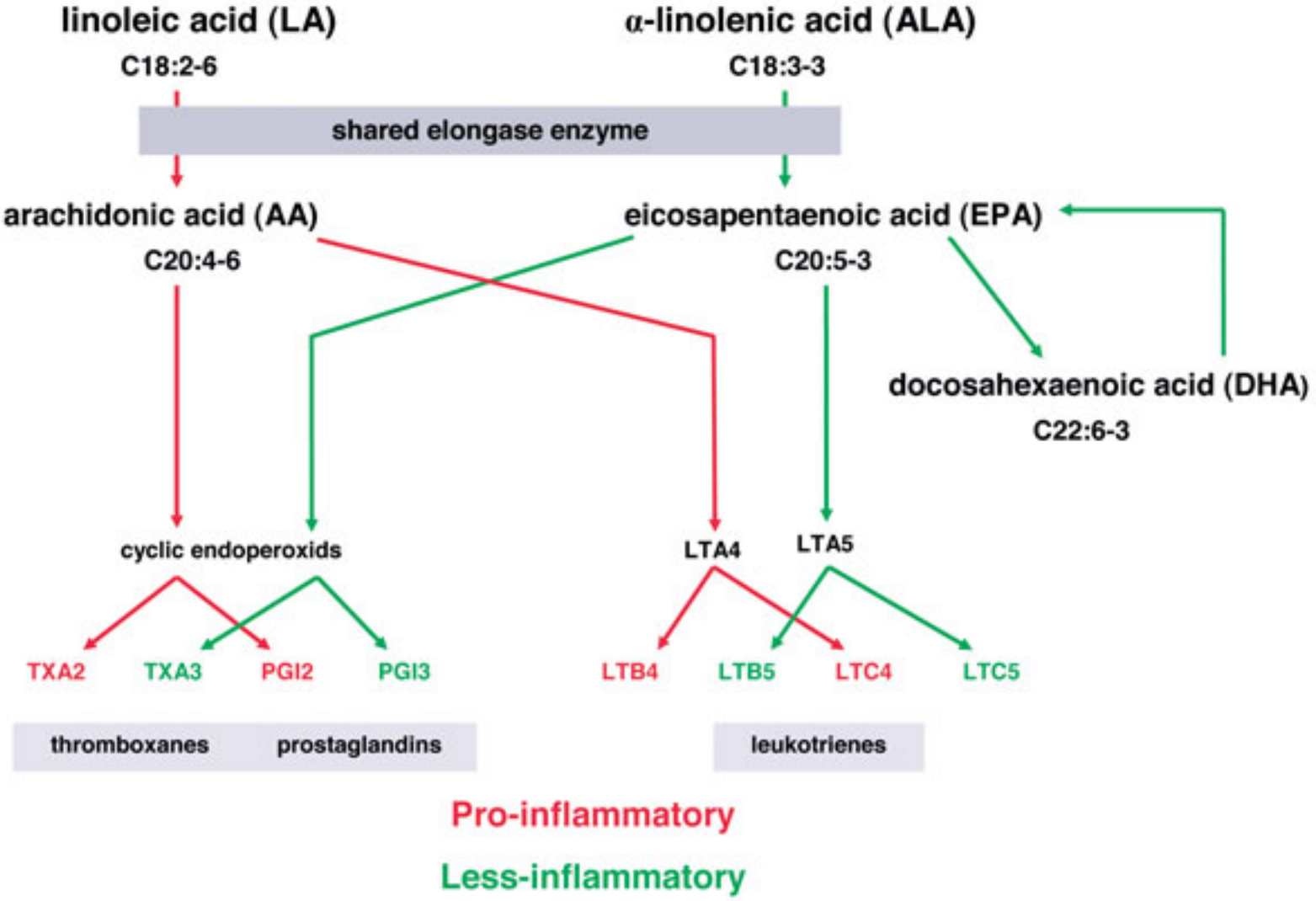


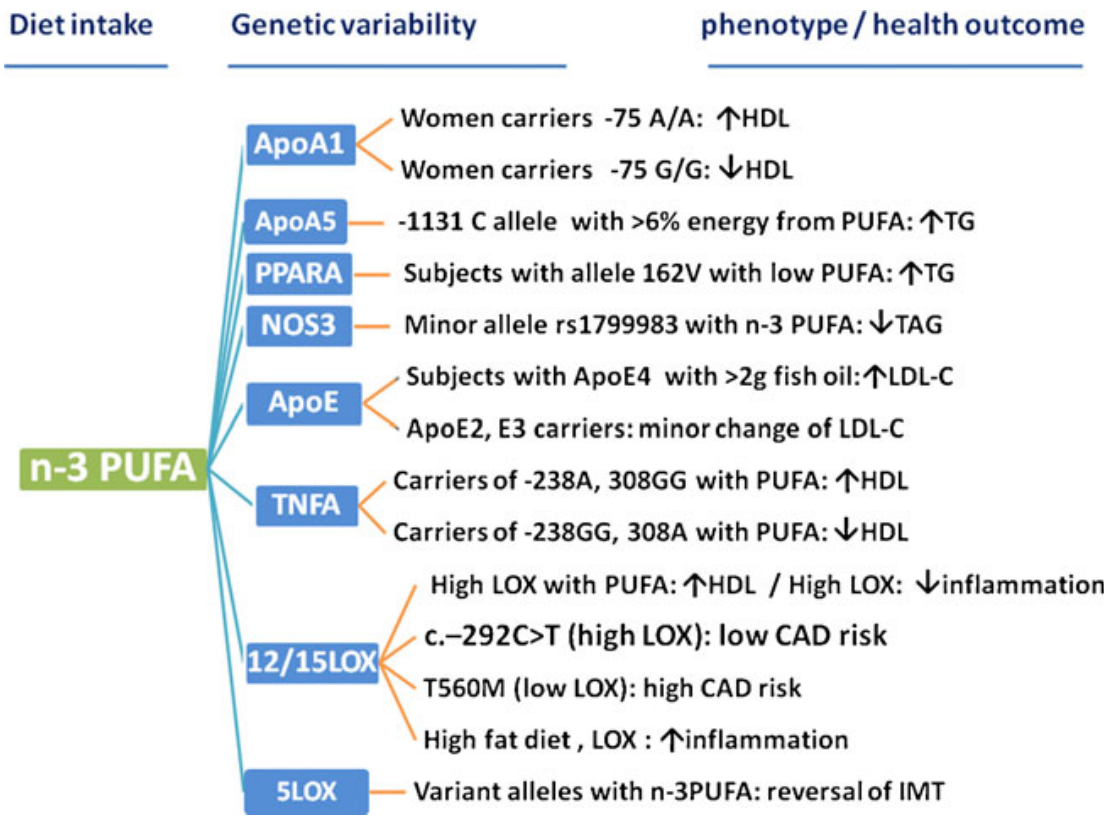
Figure 3 **Physiological Effects of n-3 PUFA That Might Influence CVD Risk**

PUFA's are generally anti-inflammatory

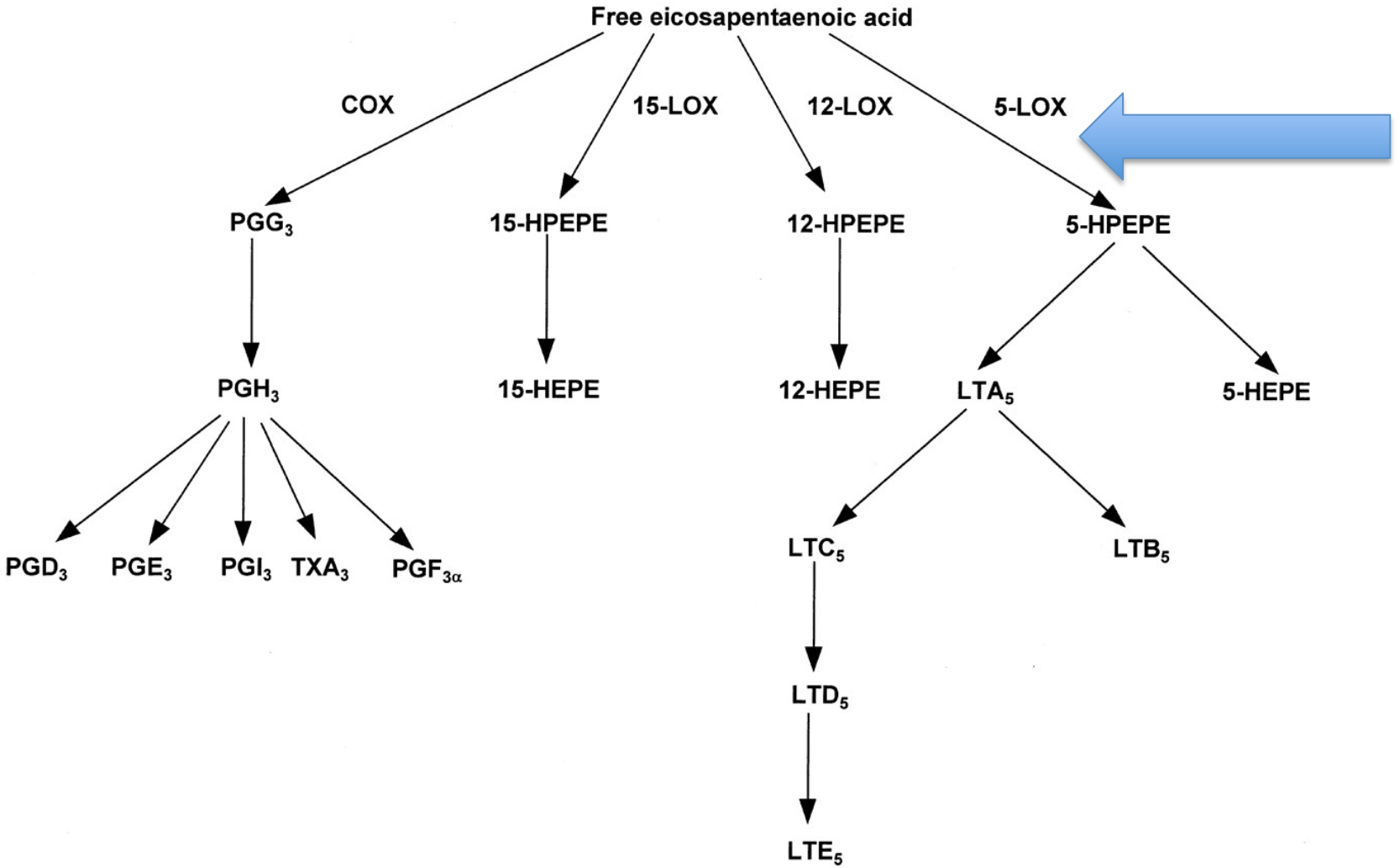


Potential Gene x Diet interactions

. 1 Nutrigenetic of omega 3
FAs in cardiovascular
ease and related traits. See
for more details



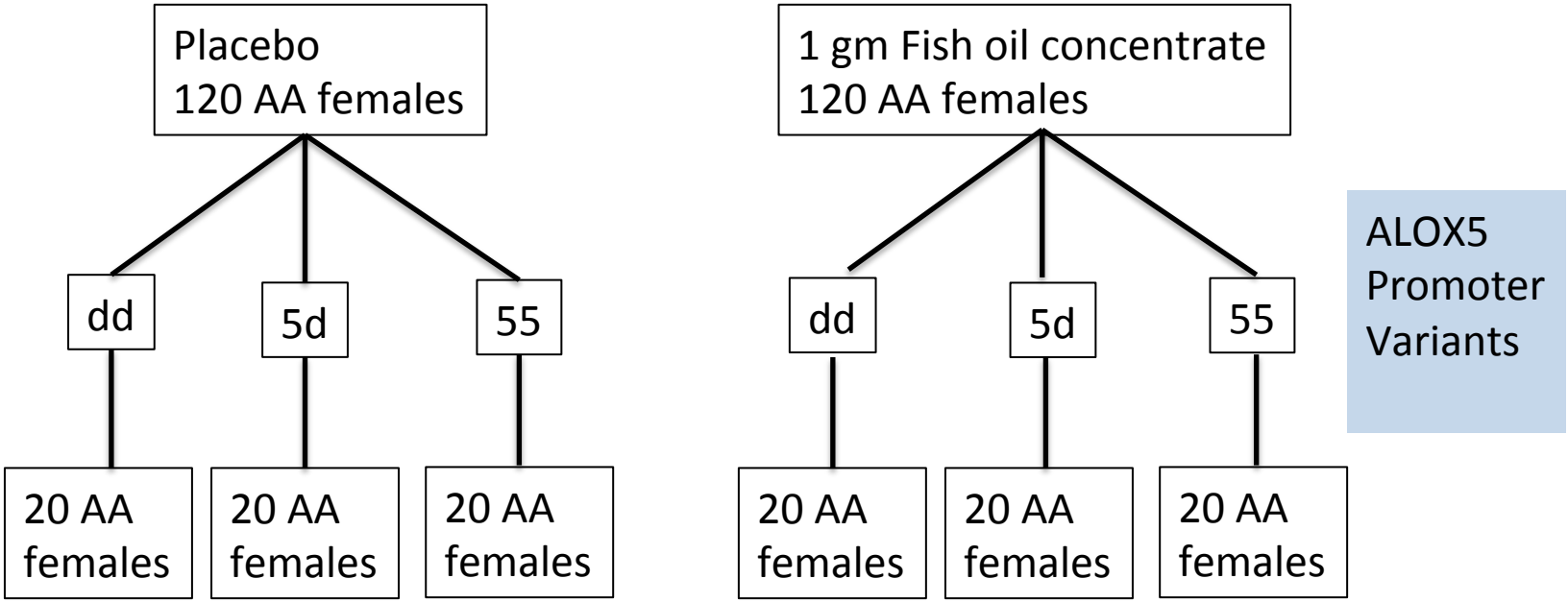
PUFA's are generally anti-inflammatory



Generalized pathway for the conversion of eicosapentaenoic acid to eicosanoids.

Philip C Calder Am J Clin Nutr 2006;83:S1505-1519S

Randomized Control Trial for NGx interactions



Charles B. Stephensen et al. J. Lipid Res. 2011;52:991-1003

five Sp1 element tandem re- peats]

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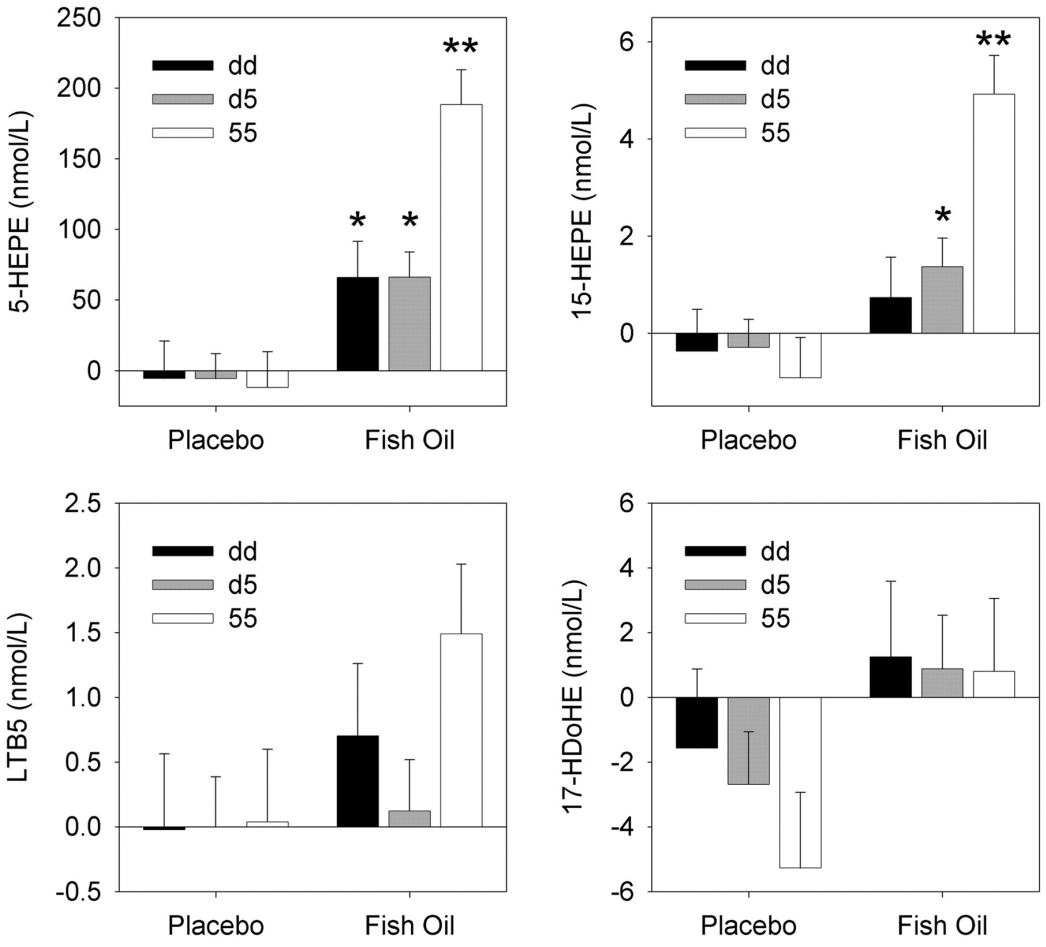
Randomized Control Trial for NGx interactions



Mean (±SEM) changes in oxylipid concentrations are shown from baseline to follow-up among the three ALOX5 genotype groups of interest in response to placebo and fish oil interventions.

Charles B. Stephensen et al. J. Lipid Res. 2011;52:991-1003

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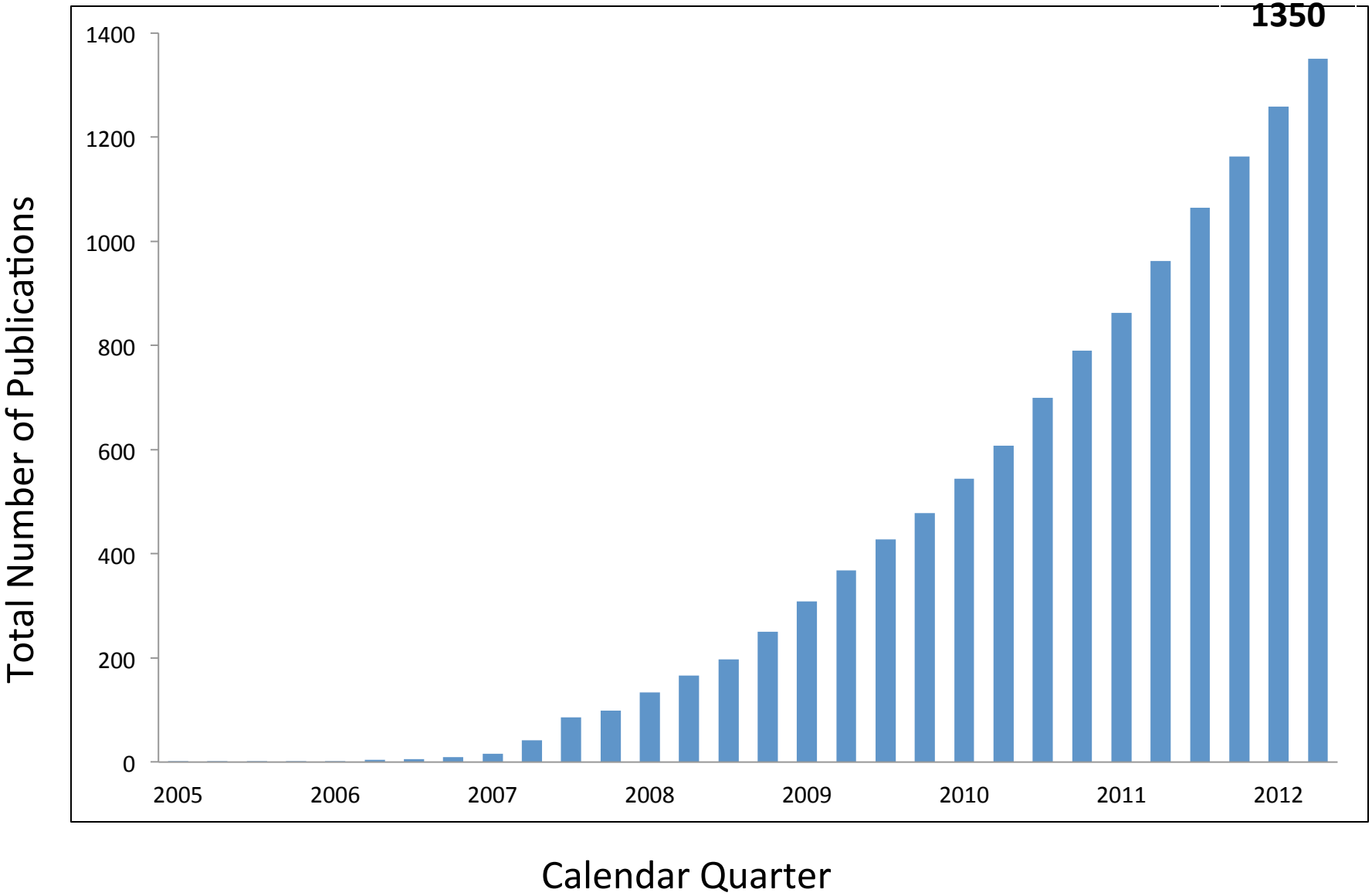
Published GWA Reports, 2005 – 6/2012



Through 6/30/12 postings

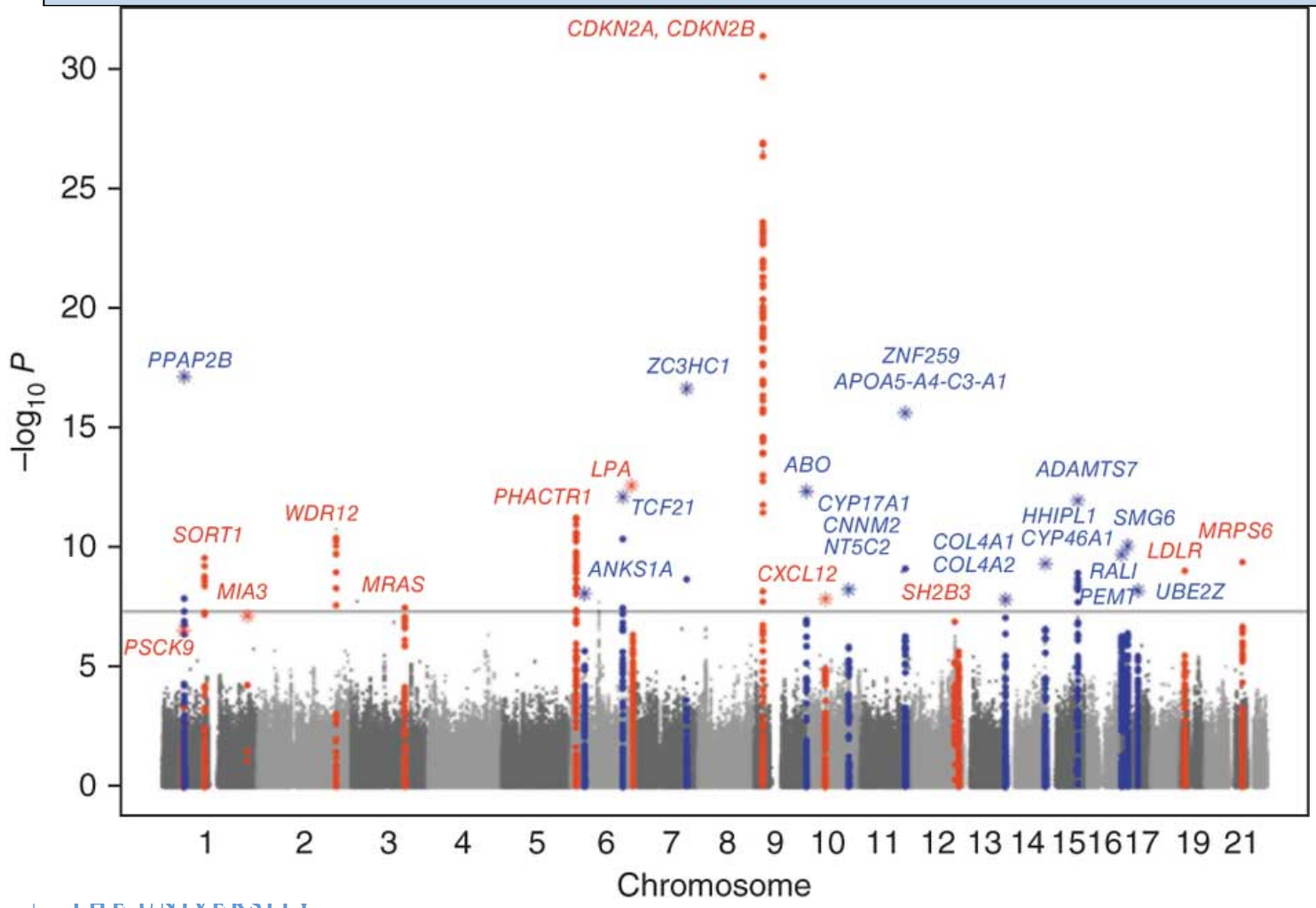
Published Genome-Wide Associations through 07/2012
Published GWA at $p \leq 5X10^{-8}$ for 18 trait categories

NHGRI GWA Catalog
www.genome.gov/GWAStudies
www.ebi.ac.uk/fgpt/gwas/

National Human Genome Research Institute

EMBL-EBI

Cardiogram results



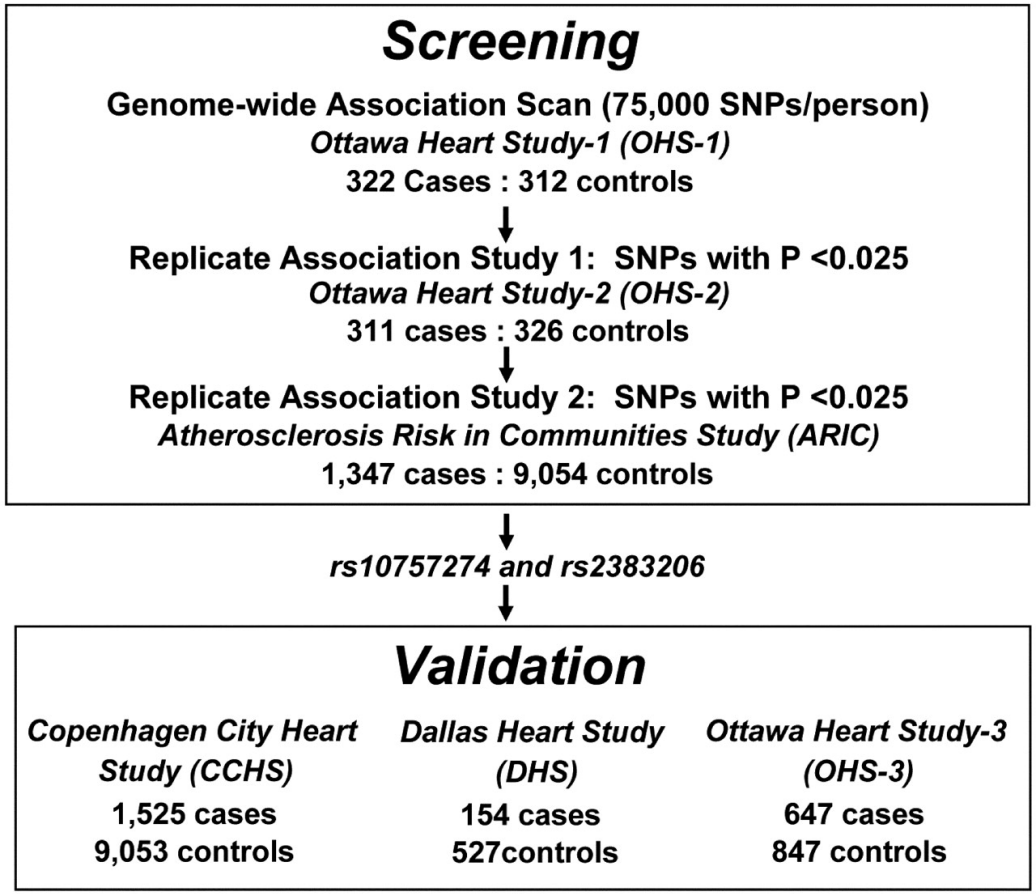
THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Initial GWAS Finding

A Common Allele on Chromosome 9 Associated with Coronary Heart Disease

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

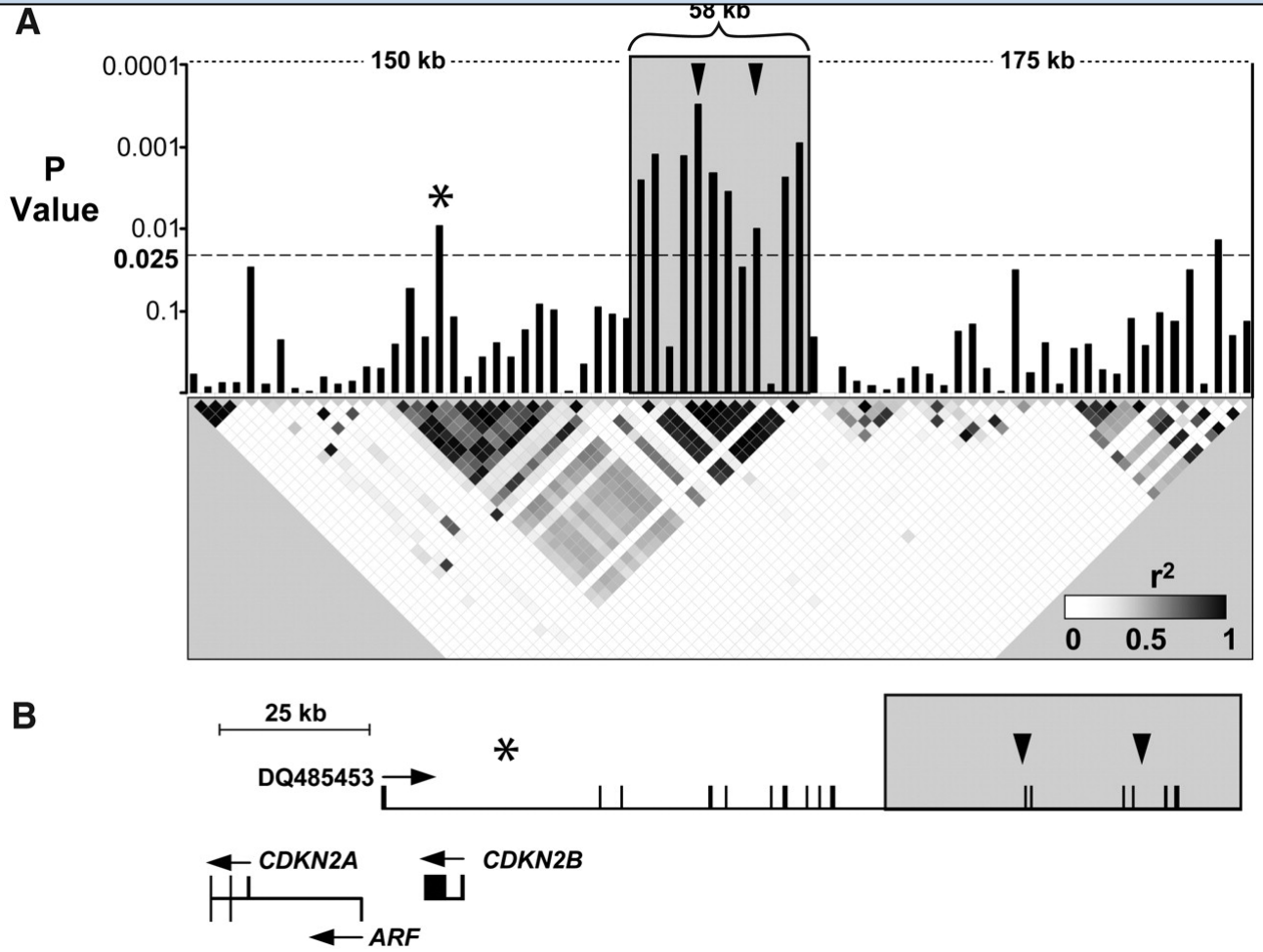
Initial GWAS Finding



Published by AAAS

R McPherson et al. Science 2007;316:1488-1491

9p21 Locus



Fine mapping of the genomic interval on chromosome 9 associated with CHD. (A) SNPs spaced ~5 kb apart in the interval extending 175 kb upstream and downstream of rs10757274 and rs2383206 were assayed in 500 cases and 500 controls from the OHS population with GeneChip Human Mapping 500K Array Sets (Affymetrix, Santa Clara, CA).

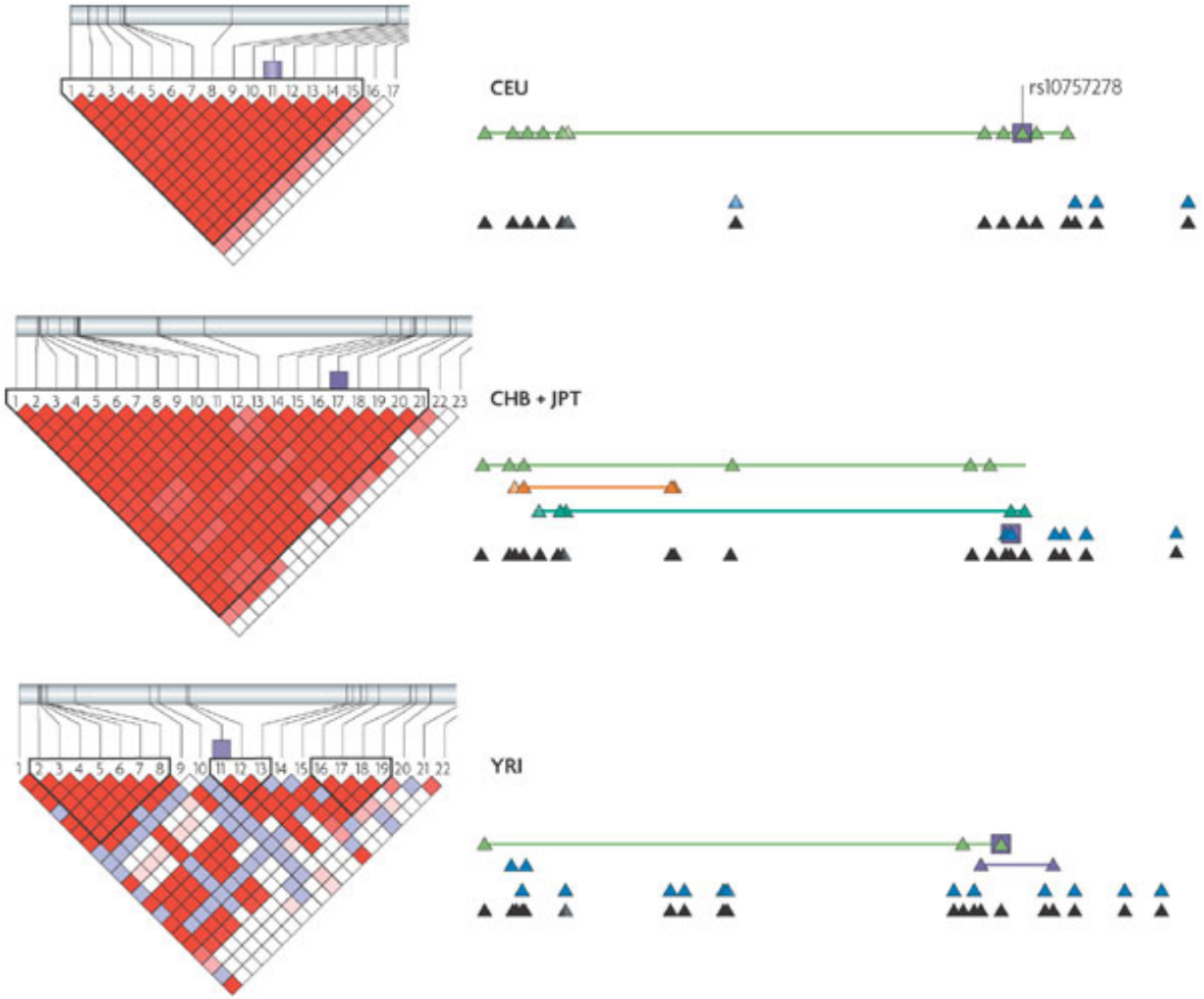
R McPherson et al. Science 2007;316:1488-1491

9p21 locus- Associations reported

Disease/Trait	Reported Gene(s)	Strongest SNP-Risk Allele	P-value	# pubs
Abdominal aortic aneurysm	CDKN2A,CKDN2B	rs2383207-G	2 x 10-8	1
Ankle-brachial index	CDKN2B	rs10757269-G	9 x 10-9	1
Breast cancer	CDKN2A,CDKN2B	rs1011970-T	3 x 10-8	1
Coronary artery calcification	CDKN2B	rs1333049-C	3 x 10-24	1
Coronary heart disease	CDKN2B-AS1	rs4977574-G	2 x 10-25	7
Endometriosis	CDKN2BAS	rs10965235-C	6 x 10-12	2
Glaucoma	CDKN2B-AS1	rs2157719-?	2 x 10-18	5
Glioma	CDKN2A, CDKN2B	rs2157719-?	5 x 10-16	4
Intracranial aneurysm	CDKN2A,CDKN2B	rs1333040-T	2 x 10-22	4
Melanoma	CDKN2A	rs7023329-A	4 x 10-7	2
Myocardial infarction	CDKN2A,CDKN2B	rs4977574-G	3 x 10-44	2
Nasopharyngeal carcinoma	CDNK2A,CDKN2B	rs1412829-?	5 x 10-7	1
Platelet counts	CDKN2A	rs3731211-A	6 x 10-14	1
Type 2 diabetes	CDKN2A, CDKN2B	rs2383208-A	2 x 10-29	12

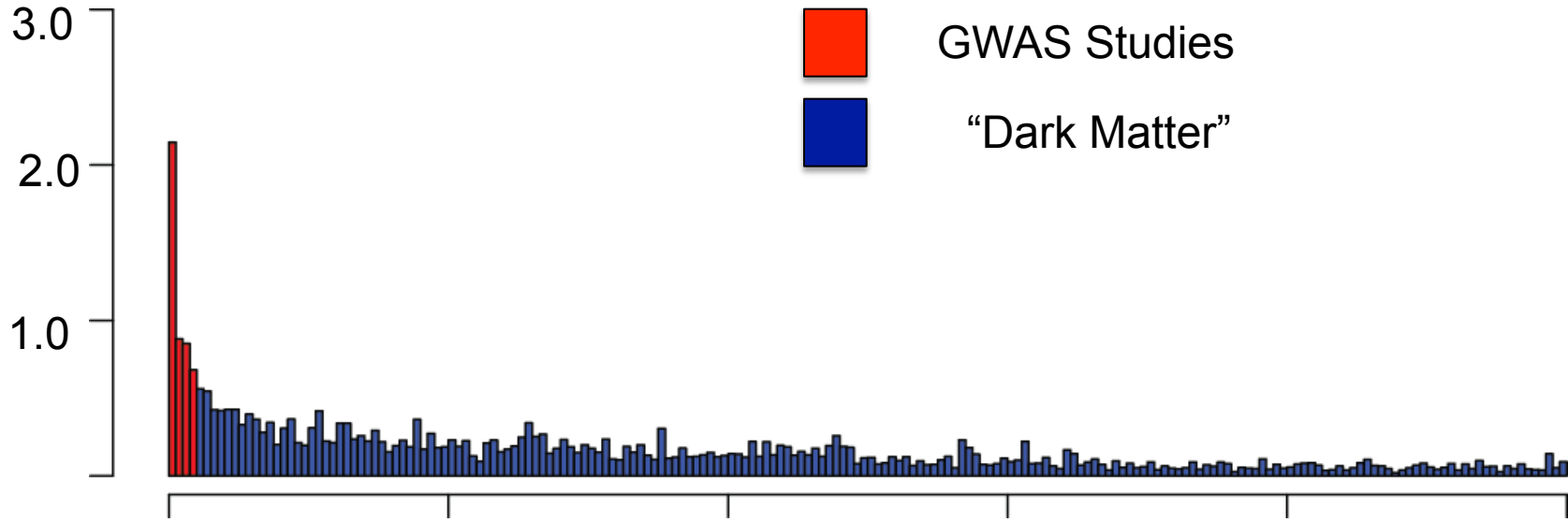
http://www.genome.gov/gwastudies/index.cfm

9p21.3 locus- structure varies by population



Kelly A. Frazer, Sarah S. Murray, Nicholas J. Schork & Eric J. Topol
Nature Reviews Genetics **10**, 241-251 (April 2009)

Missing Heritability



HDL- Heritability of HDL is 45-75%

-GWAS loci for HDL correspond to 12.1% of total variance

- 25-30% of the genetic variance

Dark matter a result of:

-over estimation of total genetic component (Lander 2012)

-gene x gene interactions

-gene x environment interactions

-environmental heterogeneity

Can 9p21 Risk Be Modified by Dietary Intake?

OPEN ACCESS Freely available online

PLoS MEDICINE

The Effect of Chromosome 9p21 Variants on Cardiovascular Disease May Be Modified by Dietary Intake: Evidence from a Case/Control and a Prospective Study

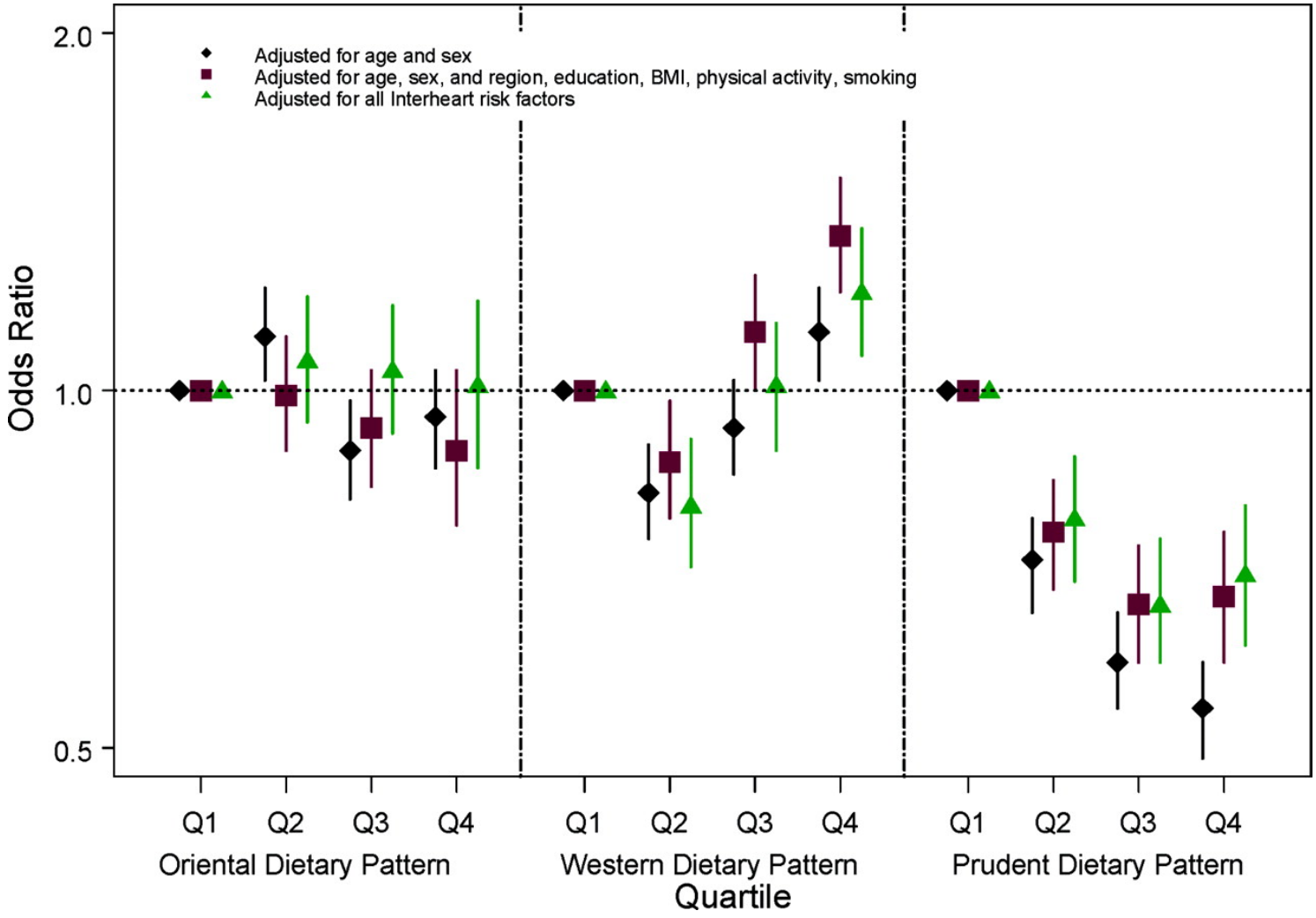
Ron Do[1], Changchun Xie[2,3], Xiaohe Zhang[2], Satu Männistö[4], Kennet Harald[4], Shofiqul Islam[2,3], Swneke D. Bailey[1], Sumathy Rangarajan[2], Matthew J. McQueen[2], Rafael Diaz[5], Liu Lisheng[6], Xingyu Wang[7], Kaisa Silander[4,8], Leena Peltonen[4,8†], Salim Yusuf[2], Veikko Salomaa[4], James C. Engert[1,9,10*], Sonia S. Anand[2,3*], on behalf of the INTERHEART investigators

- **Five** ethnicities
- Retrospective **Case-Control study** → Risk factors for acute non-fatal MI
- Recruitment → within24 hours of being admitted to a coronary care unit with clinical characteristics of acute MI. → Control matched for age and sex.

Four SNPs (rs10757274, **rs2383206**, rs10757278, rs1333049) from the Chromosome 9p21 region were selected based on previous results from genome-wide association studies for coronary heart disease/MI

Genotypes : 1,744 Europeans, 1,867 South Asians, 2,231 Chinese, 1,100 Latin Americans, and 1,172 Arabs (a total of 8,114 samples).

Risk of MI associated with quartiles of dietary patterns (95% CI).



Romaina Iqbal et al. Circulation. 2008;118:1929-1937

American Heart Association.

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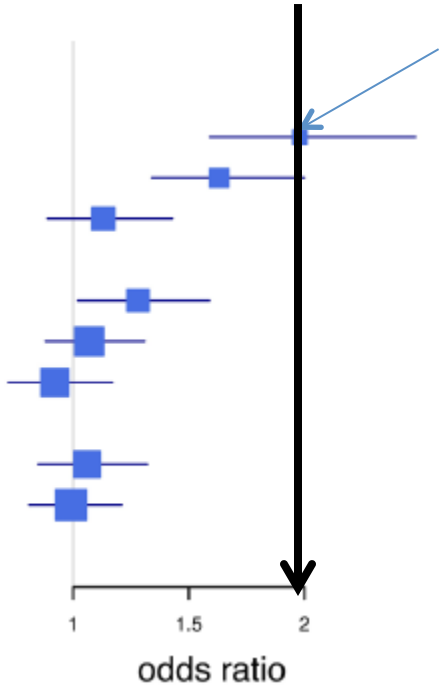
The Effect of a Prudent Diet is most pronounced in individuals with Risk Allele

rs2383206	n	OR	CI	p
Low group of prudent diet				
GG	800	1.98	1.59–2.48	2.11e–9
AG	1284	1.63	1.34–2.00	1.65e–6
AA	561	1.13	0.89–1.43	0.32
Medium group				
GG	830	1.28	1.02–1.59	0.032
AG	1279	1.07	0.88–1.31	0.51
AA	524	0.92	0.72–1.17	0.51
High group				
GG	825	1.06	0.85–1.32	0.62
AG	1241	0.99	0.81–1.21	0.93
AA	573	1		

rs2383206



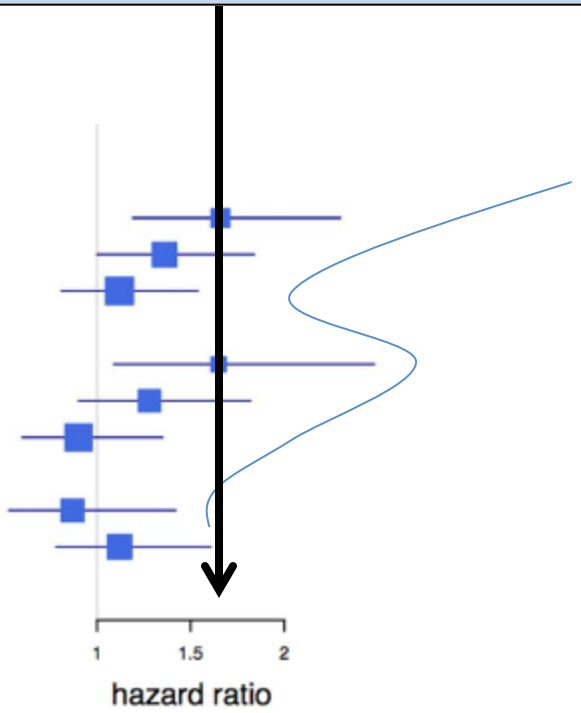
Will Dietary interventions be most effective in these people

INTERHEART

An analysis of the rs2383206 genotype and tertiles of the prudent diet score in the INTERHEART samples demonstrated that individuals with two copies of the risk allele (GG) and with a low prudent diet score had a ,2-fold increase in MI risk when compared to the reference group of individuals with two copies of the protective allele (AA) and a high prudent diet score.

The Effect of a Prudent Diet is most pronounced in individuals with Risk Allele

rs4977574	n	n case	HR	CI	p
Low group of prudent diet					
GG	2270	136	1.66	1.19–2.30	2.6e–3
AG	5978	333	1.36	1.00–1.84	0.053
AA	4066	209	1.12	0.81–1.54	0.49
Medium group					
GG	605	47	1.65	1.09–2.48	0.012
AG	1762	91	1.28	0.90–1.82	0.16
AA	1159	51	0.90	0.60–1.35	0.60
High group					
GG	620	24	0.87	0.53–1.42	0.57
AG	1611	76	1.12	0.78–1.61	0.54
AA	1058	47	1		



The combination of a diet low in fruits, berries, and vegetables and two copies of the risk allele was associated with a 1.66-fold increase in risk for CVD but no consistent effect of the 9p21 SNP was observed in the group with the most prudent diet.

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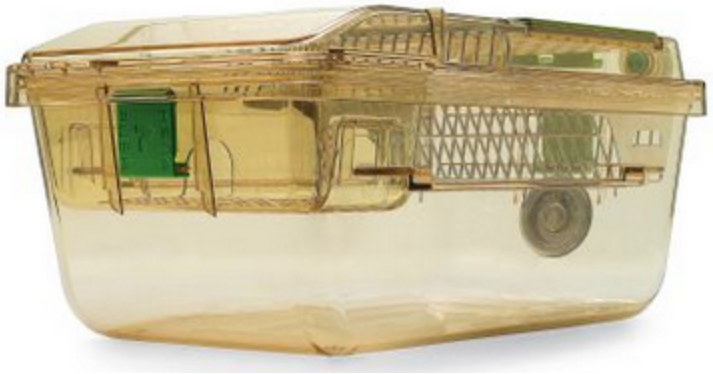
Why Mice for Genetic Studies?



Tightly controlled environment

- Housed in standardized cages
- Given synthetically defined diets
- Regulate access to food

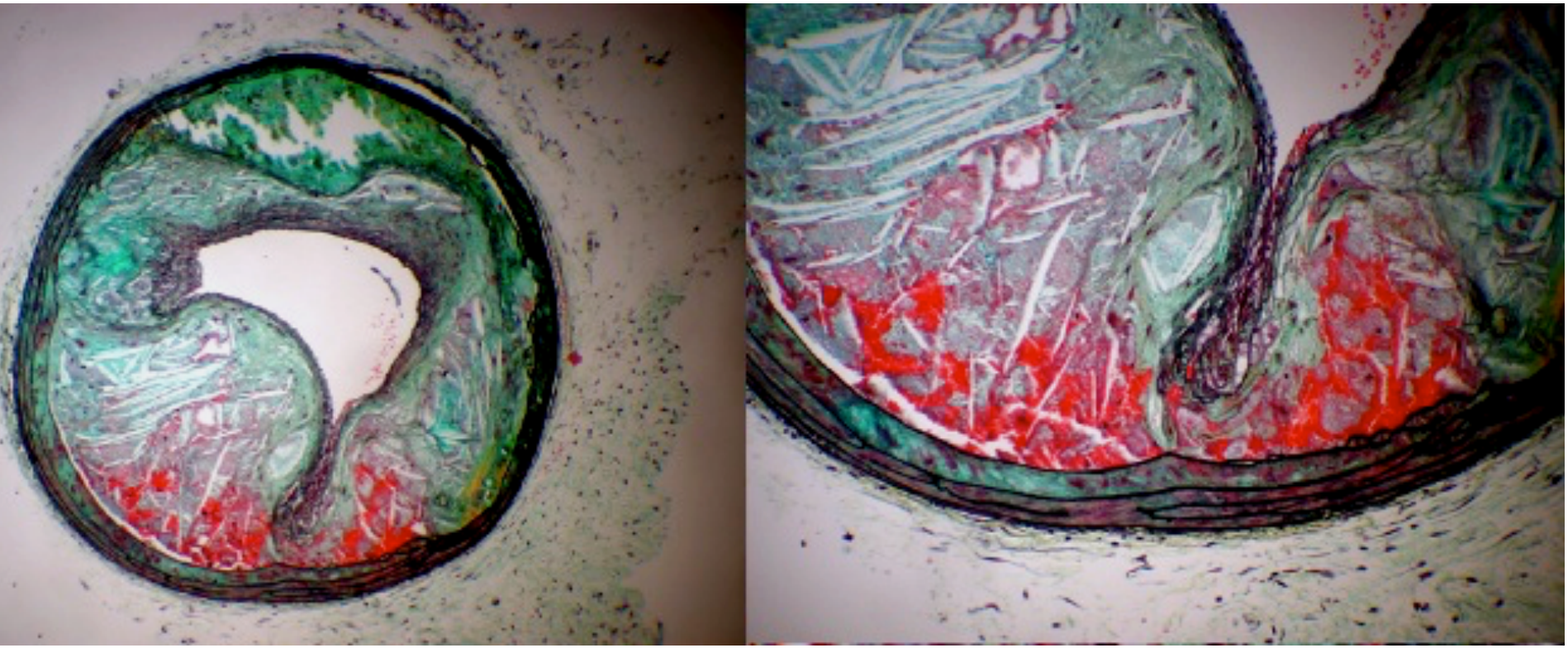
Resistant?

Susceptible?

Focus on genetic differences

- Identify genes regulating the atherosclerosis

Advanced atherosclerosis in mice



Mice can model complex pathology that is similar to human disease

Why Mice for Genetic Studies?

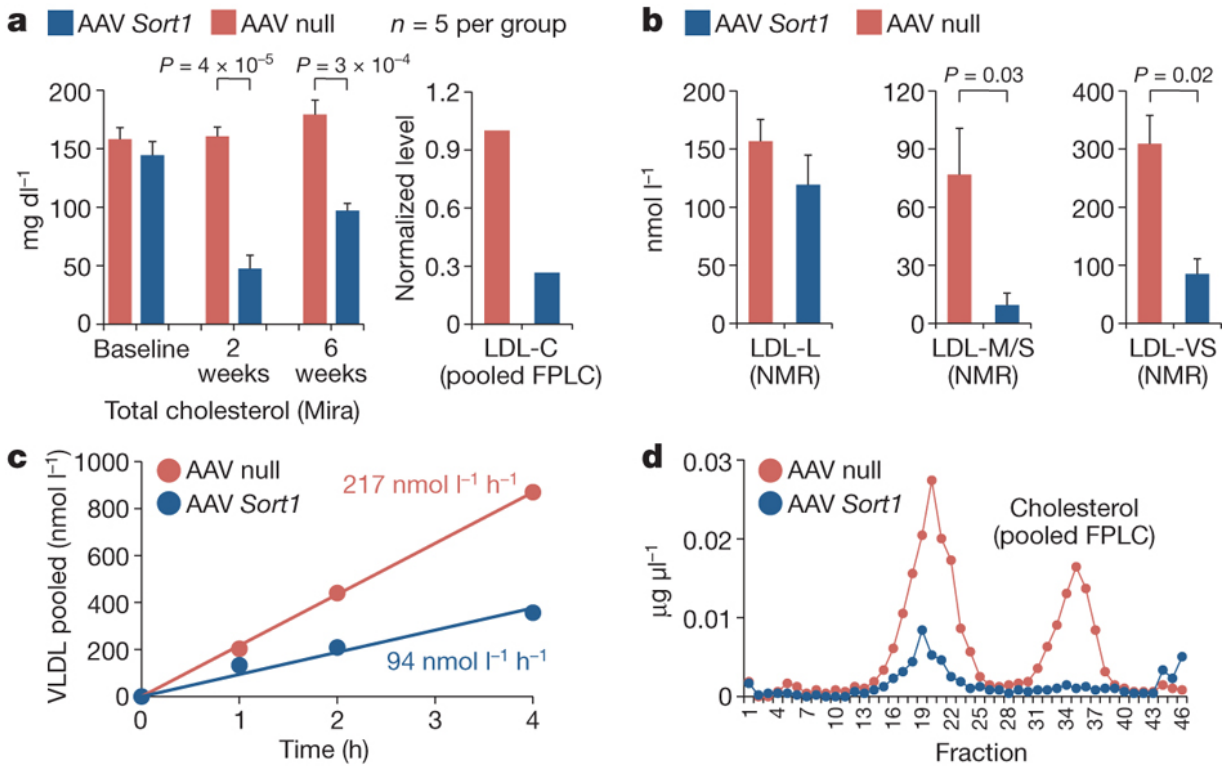
ARTICLES

From noncoding variant to phenotype via *SORT1* at the 1p13 cholesterol locus

Kiran Musunuru[1,2,3]*, Alanna Strong[4]*, Maria Frank-Kamenetsky[5], Noemi E. Lee[1], Tim Ahfeldt[1,6], Katherine V. Sachs[4], Xiaoyu Li[4], Hui Li[4], Nicolas Kuperwasser[1], Vera M. Ruda[1], James P. Pirruccello[1,2], Brian Muchmore[7], Ludmila Prokunina-Olsson[7], Jennifer L. Hall[2,8], Eric E. Schadt[9], Carlos R. Morales[10], Sissel Lund-Katz[11], Michael C. Phillips[11], Jamie Wong[5], William Cantley[5], Timothy Racie[5], Kenechi G. Ejebe[1,2], Marju Orho-Melander[12], Olle Melander[12], Victor Koteliansky[5], Kevin Fitzgerald[5], Ronald M. Krauss[13], Chad A. Cowan[1,2], Sekar Kathiresan[1,2]* & Daniel J. Rader[4]*

Recent genome-wide association studies (GWASs) have identified a locus on chromosome 1p13 strongly associated with both plasma low-density lipoprotein cholesterol (LDL-C) and myocardial infarction (MI) in humans. Here we show through a series of studies in human cohorts and human-derived hepatocytes that a common noncoding polymorphism at the 1p13 locus, rs12740374, creates a C/EBP (CCAAT/enhancer binding protein) transcription factor binding site and alters the hepatic expression of the *SORT1* gene. With small interfering RNA (siRNA) knockdown and viral overexpression in mouse liver, we demonstrate that *Sort1* alters plasma LDL-C and very low-density lipoprotein (VLDL) particle levels by modulating hepatic VLDL secretion. Thus, we provide functional evidence for a novel regulatory pathway for lipoprotein metabolism and

Modulation of *Sort1* alters plasma lipids and lipoproteins.



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nature

Approach: Systems genetics

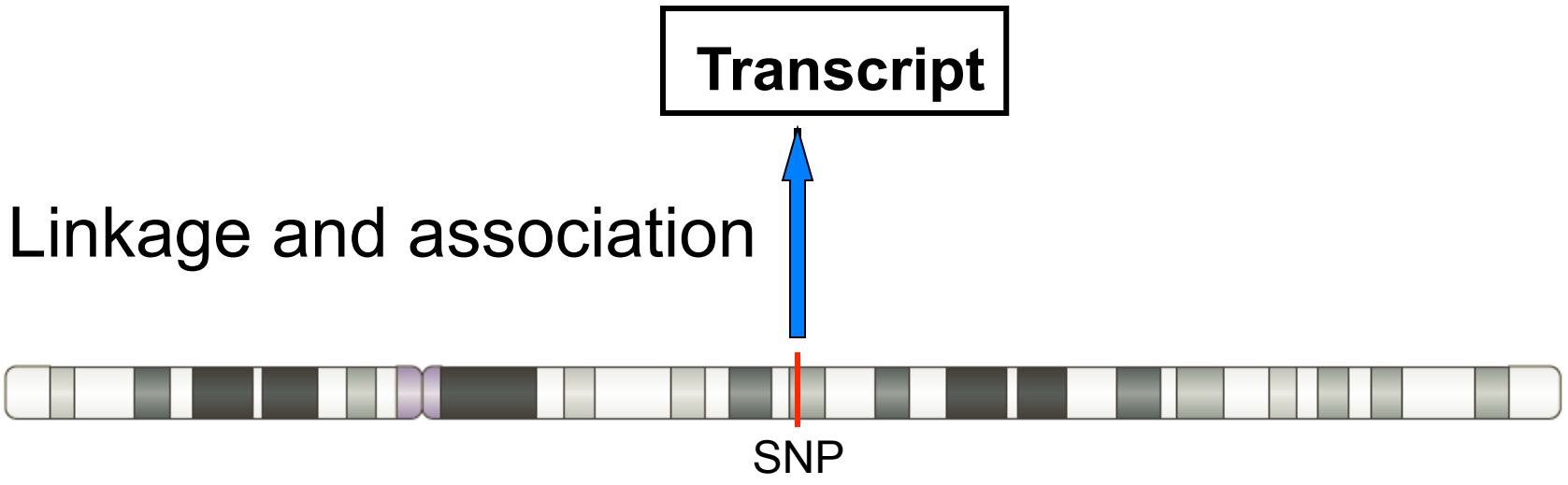
Systems genetics

- Quantitative
- High throughput ("omics") technologies

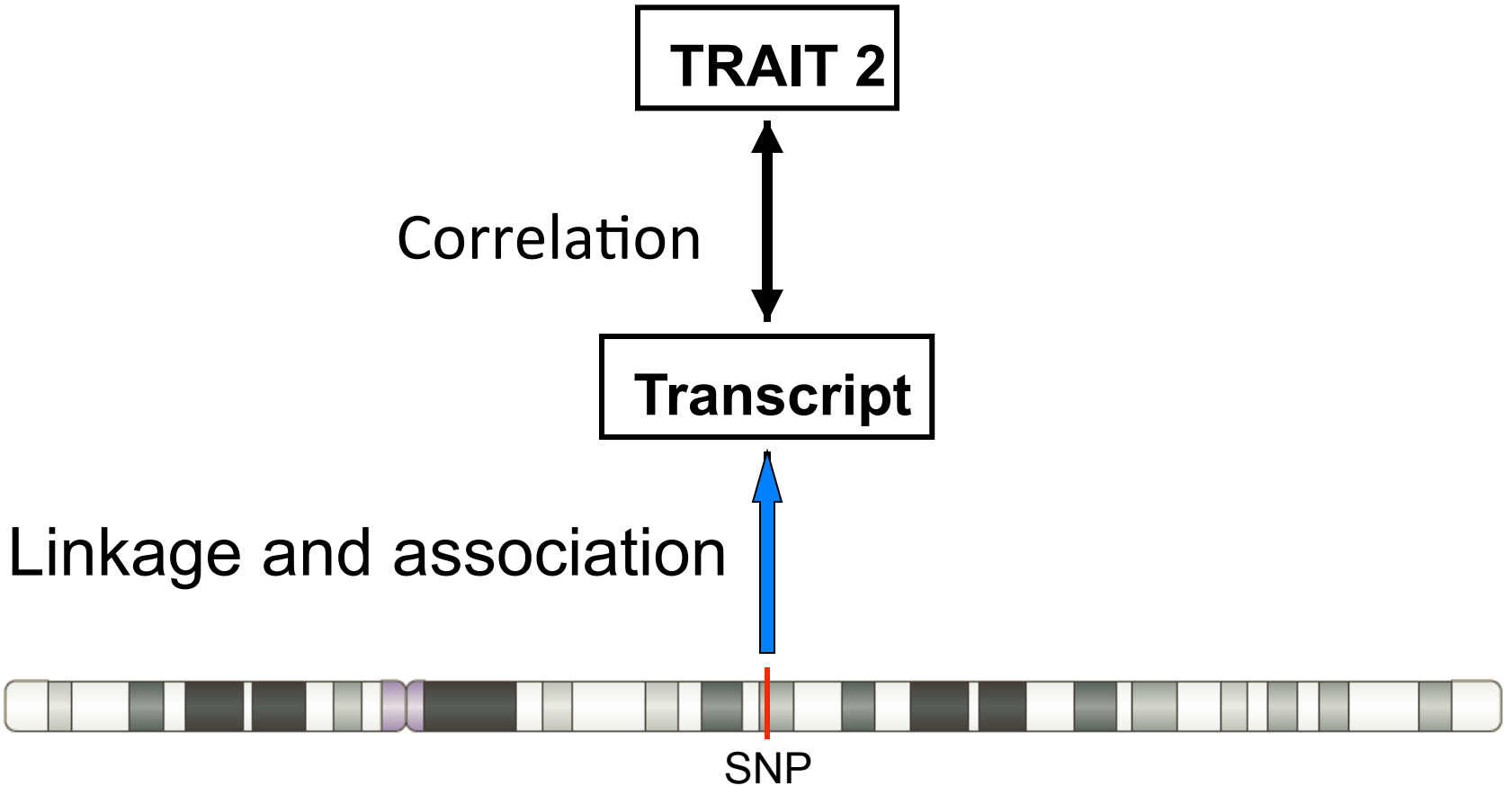
- Natural variation
- Multiple perturbations

Arabidopsis: Jensen
Yeast: Kruglyak
Drosophila: Mackay
Rats: Aitman, Cook
Mice: Williams, Lusis, Churchill
Humans: Schadt, Bjorkegren

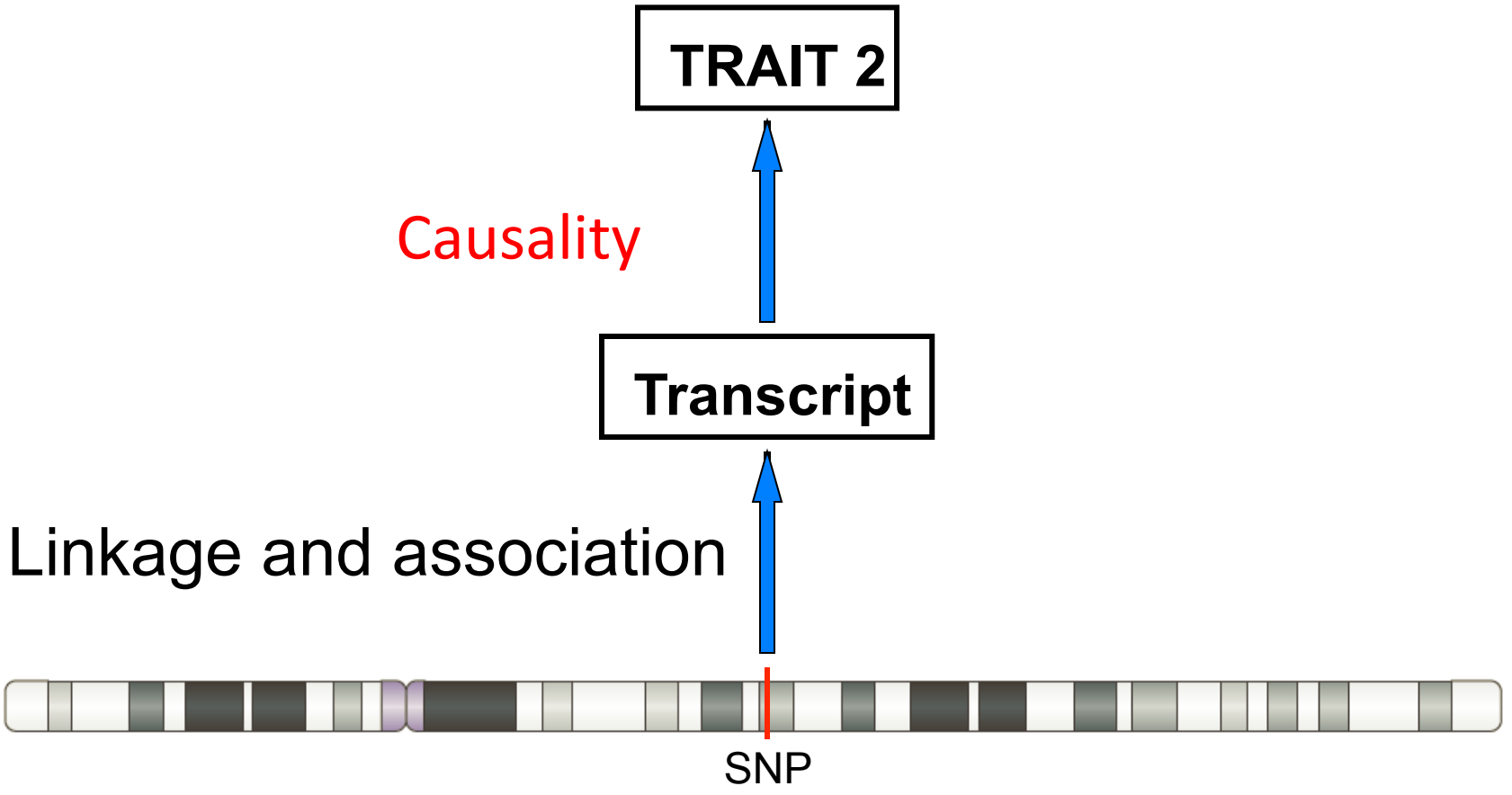
Approach: Systems genetics



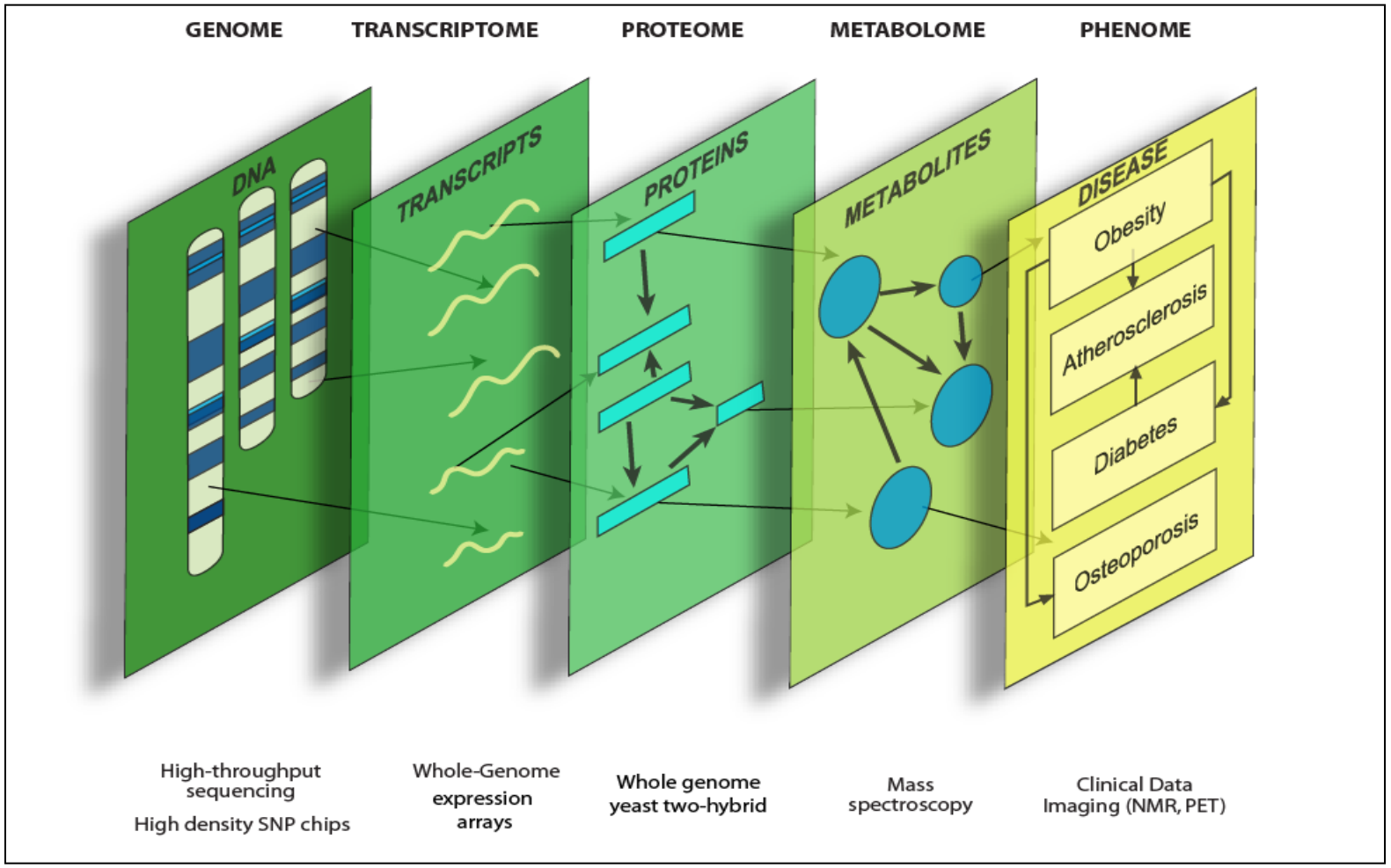
Approach: Systems genetics



Approach: Systems genetics



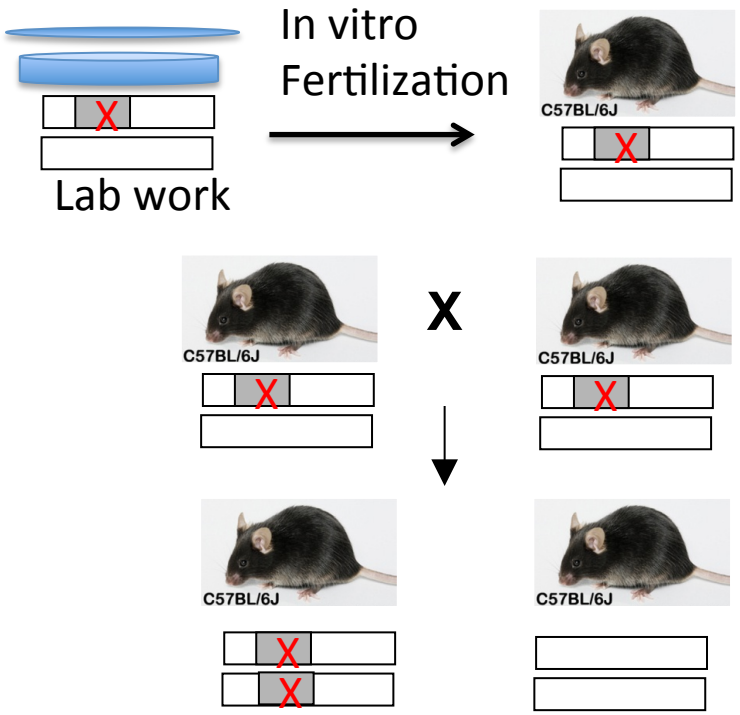
Approach: Systems genetics



Global gene expression, proteomic or metabolomic data can be integrated with genetic and phenotypic data to identify genes and pathways contributing to diseases

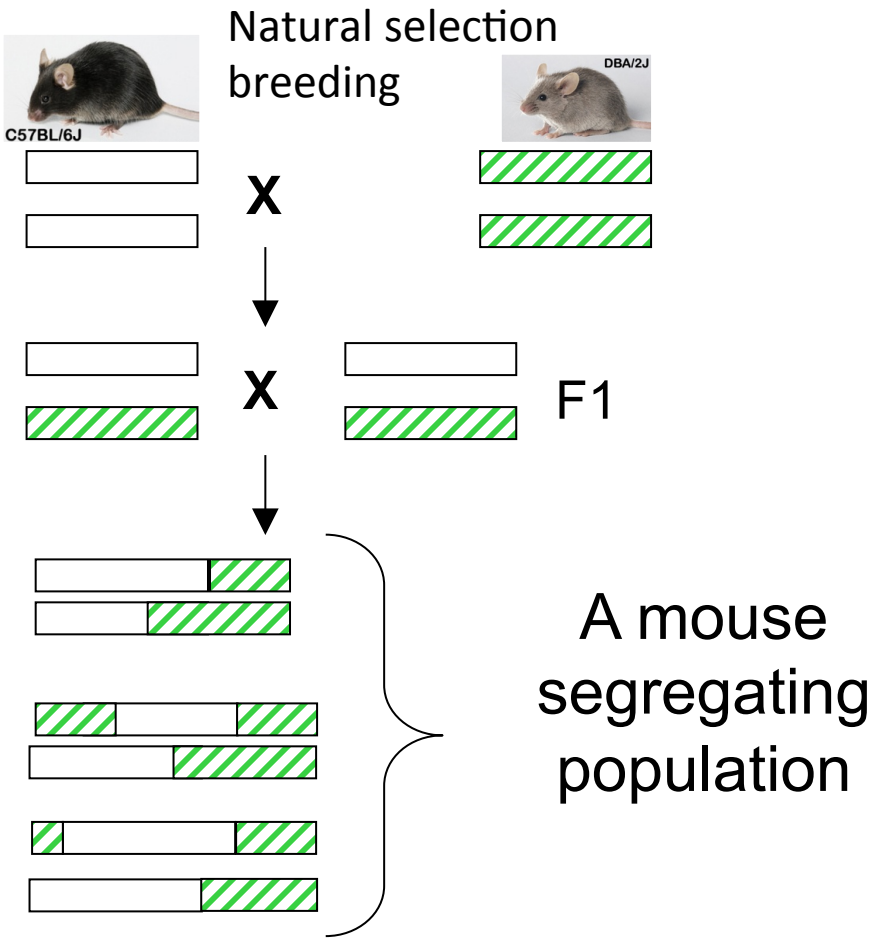
Mouse Molecular Genetics

Reverse Genetics



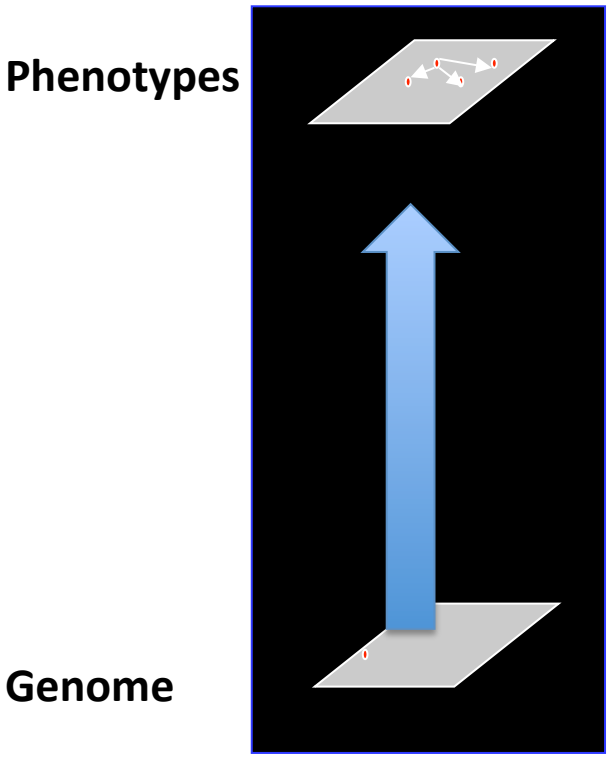
"Knockout" Studies

Forward Genetics

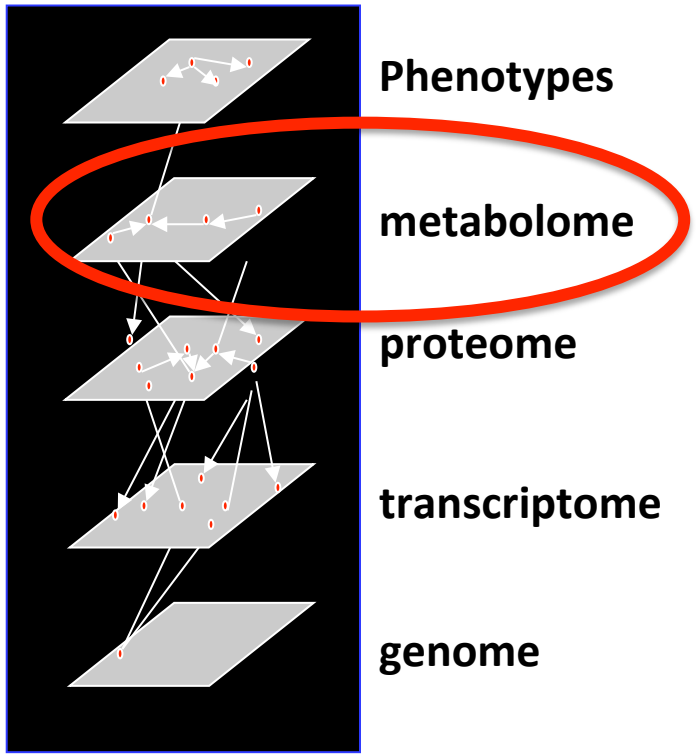


"QTL" Studies

Approach: Systems genetics

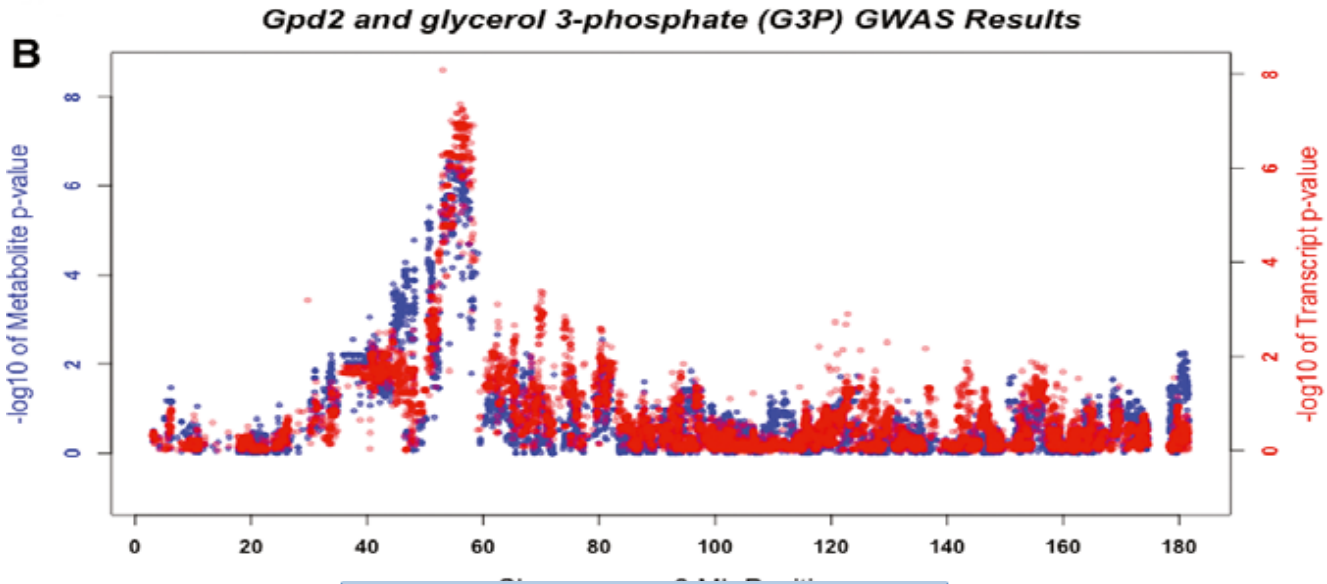


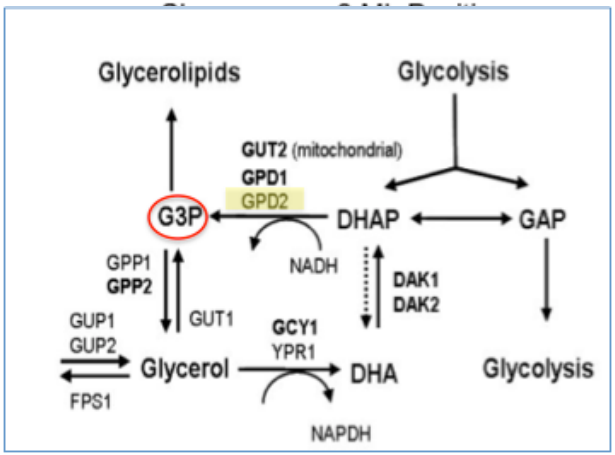
Classic Genetics



Systems Genetics

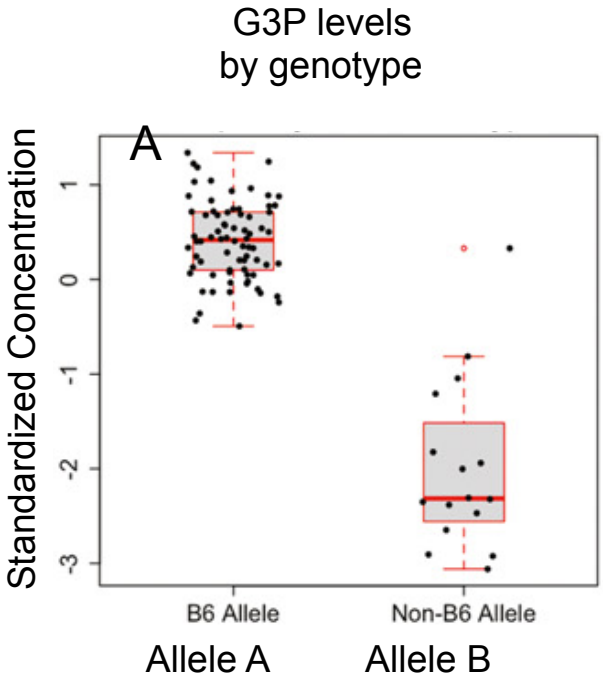
Metabolomics
Coincident mapping- shared genetic regulation

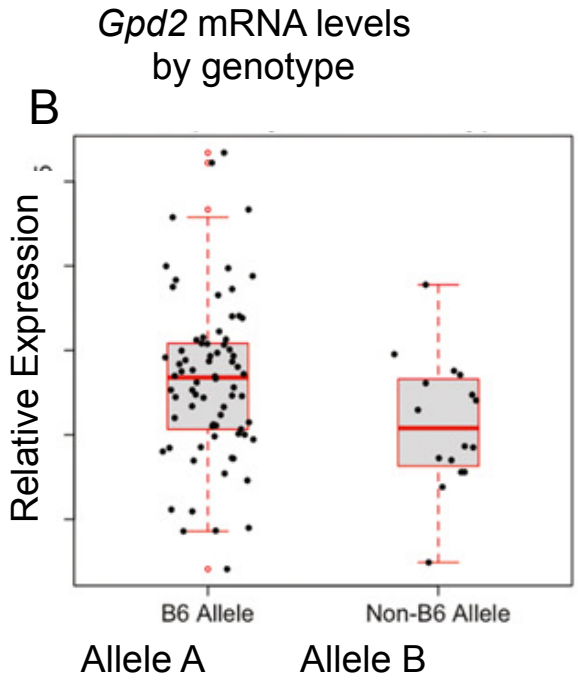


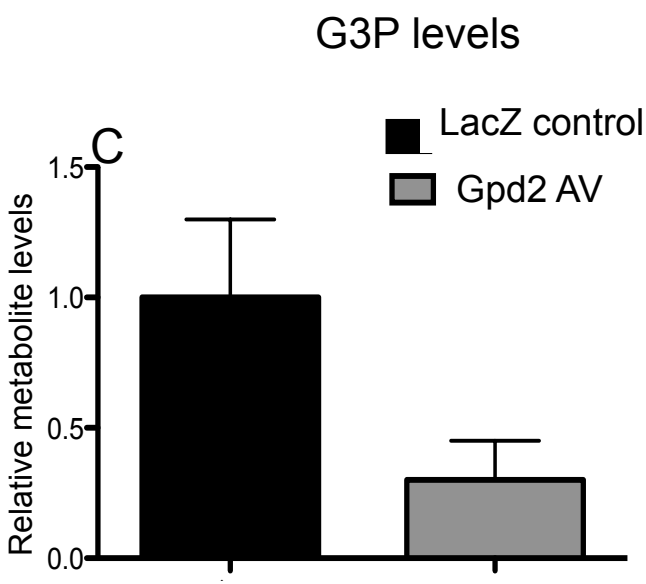


Ghazalpour, Bennett et al 2014

Metabolomics
Validation of metabolite/mRNA mapping results in the HMDP







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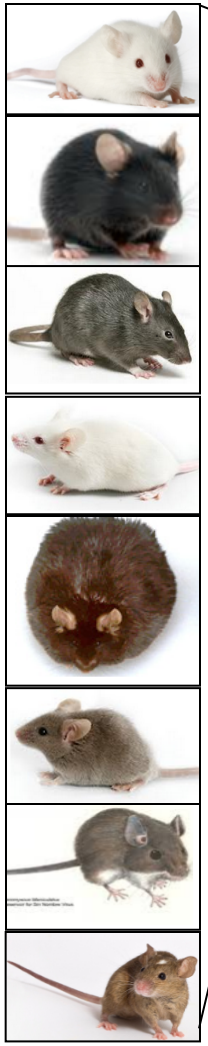
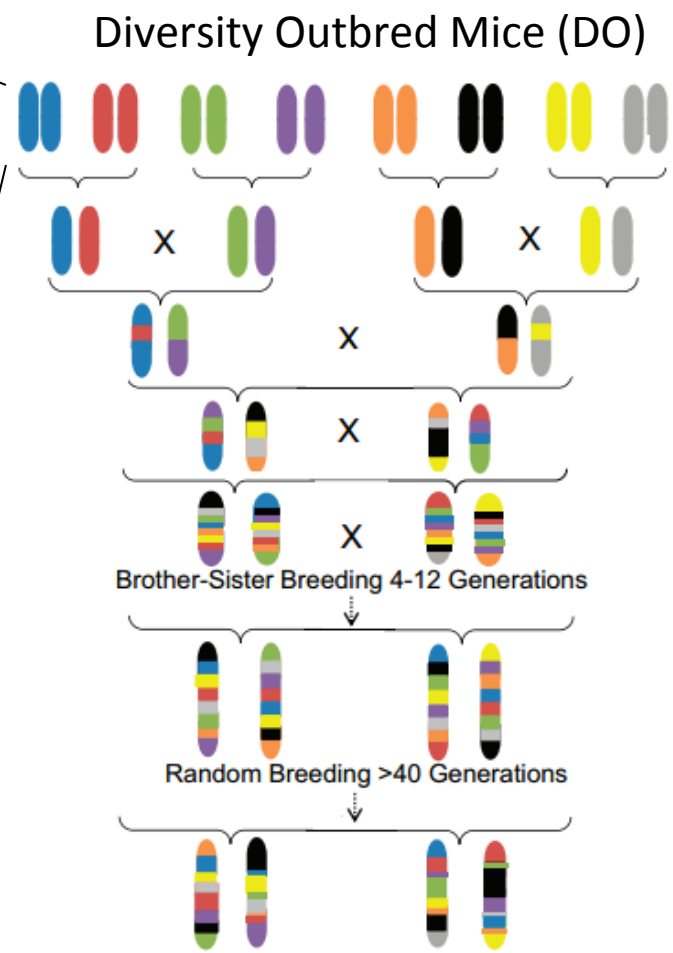
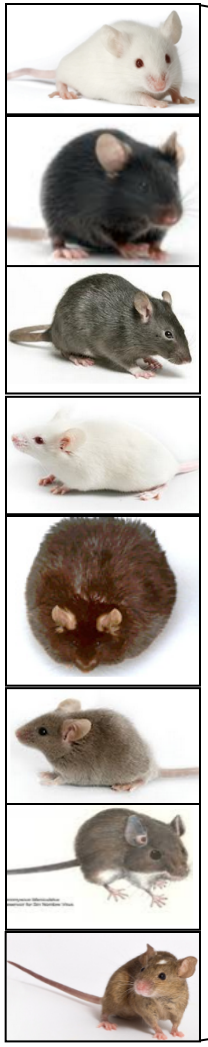
Diversity Outbred mice:
A New multi parent genetic reference panel





- These mice are derived from the 8 founders used to create the Collaborative Cross (CC).
- 144 pairs of pre-CC mice at F4-12 of inbreeding were used to create the DO.
- While the CC are maintained as RILs, DO are maintained by randomized outbreeding

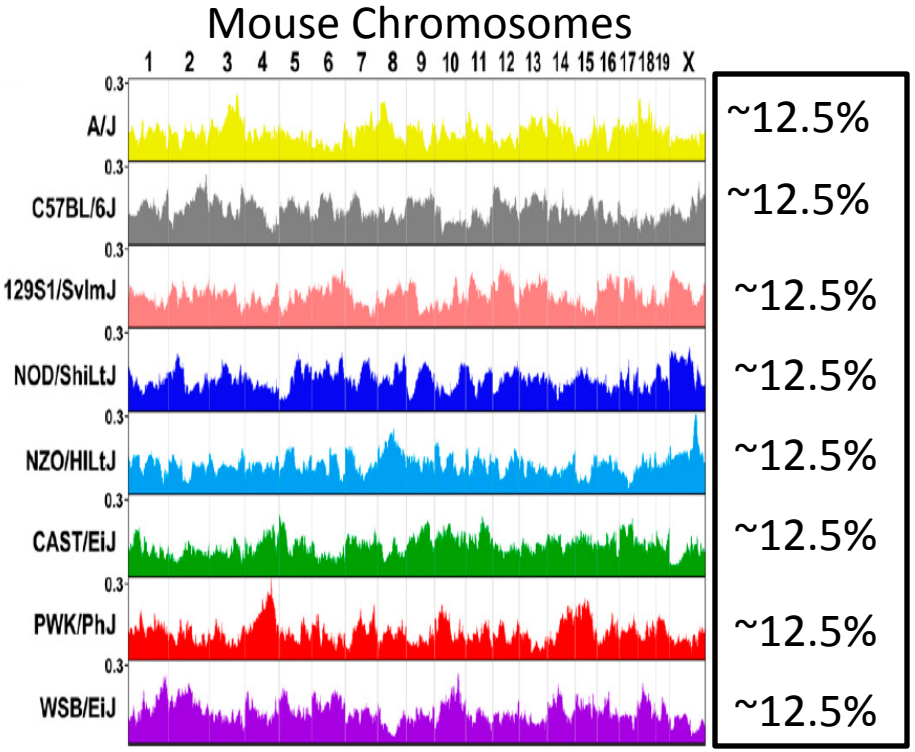
A new multi parent mouse resource has been designed to improve QTL mapping resolution



Strain Name	Letter	Color
A/J	A	
C57BL/6J	B	
129S1/SvImJ	C	
NOD/ShiLtJ	D	
NZO/HiLtJ	E	
CAST/EiJ	F	
PWK/PhJ	G	
WSB/EiJ	H	

Diversity Outbred mice

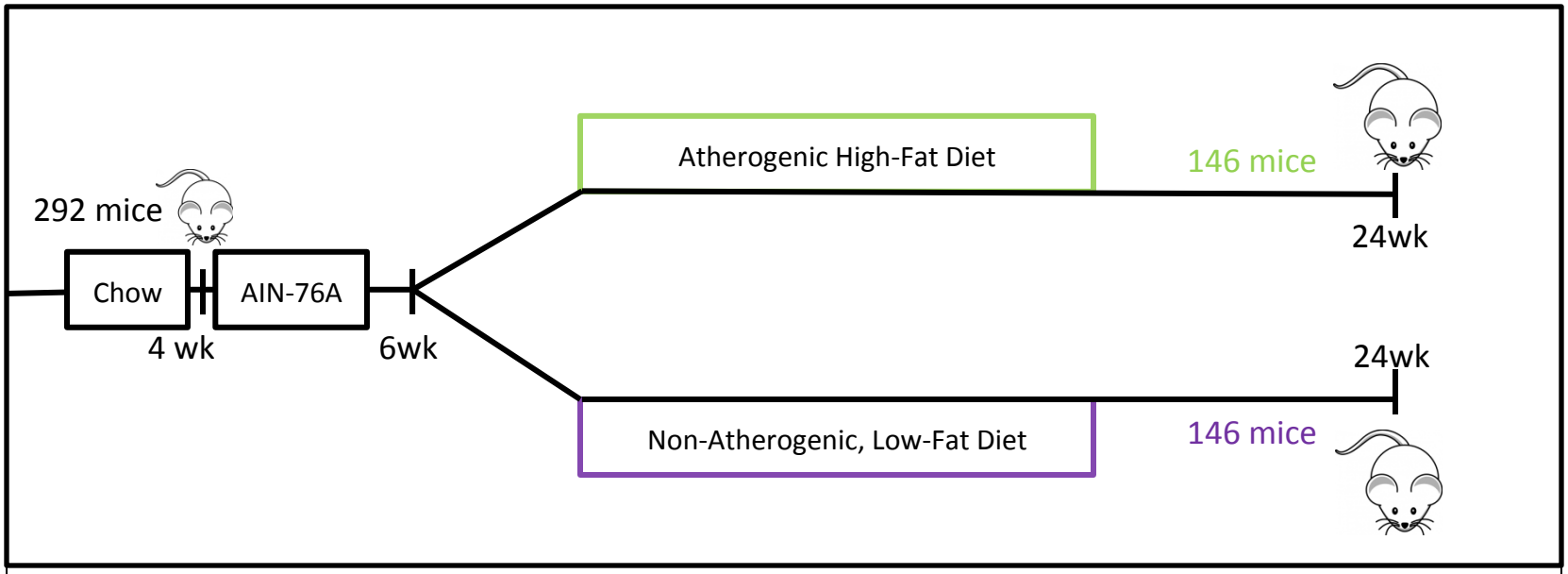
Combination of 8 inbred founders results in genetically diverse population



Total genome of DO animal = 100%

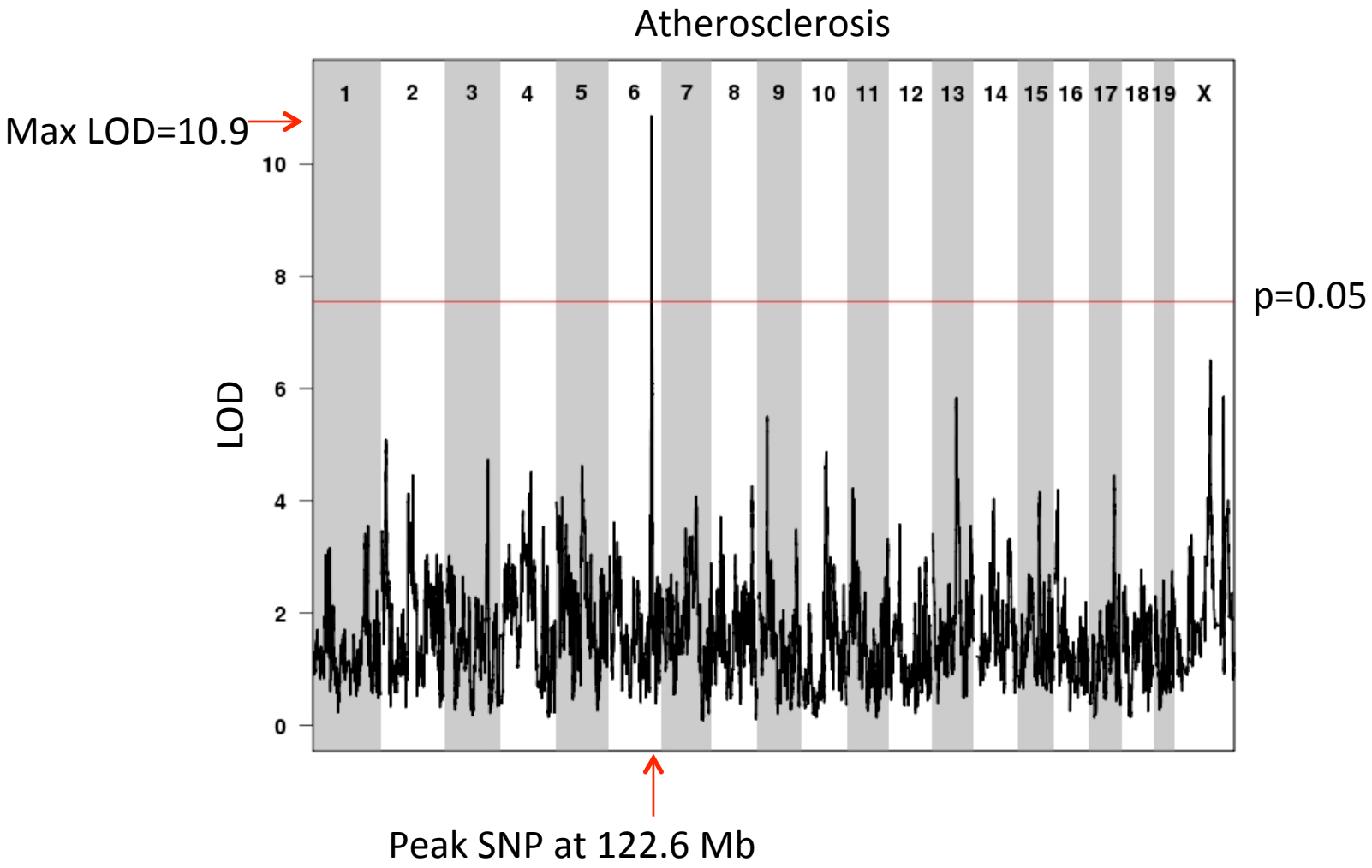
- These strains capture 90% of the known allelic diversity across inbred strains.
- There are ~45 million polymorphic loci between the 8 founder strains
  - 37.8 million SNPs
  - 6.9 million insertions, deletions, structural variants
- Each DO mouse has roughly equal founder allele contribution across the genome from each inbred strain.

Diversity Outbred mice: Study Design



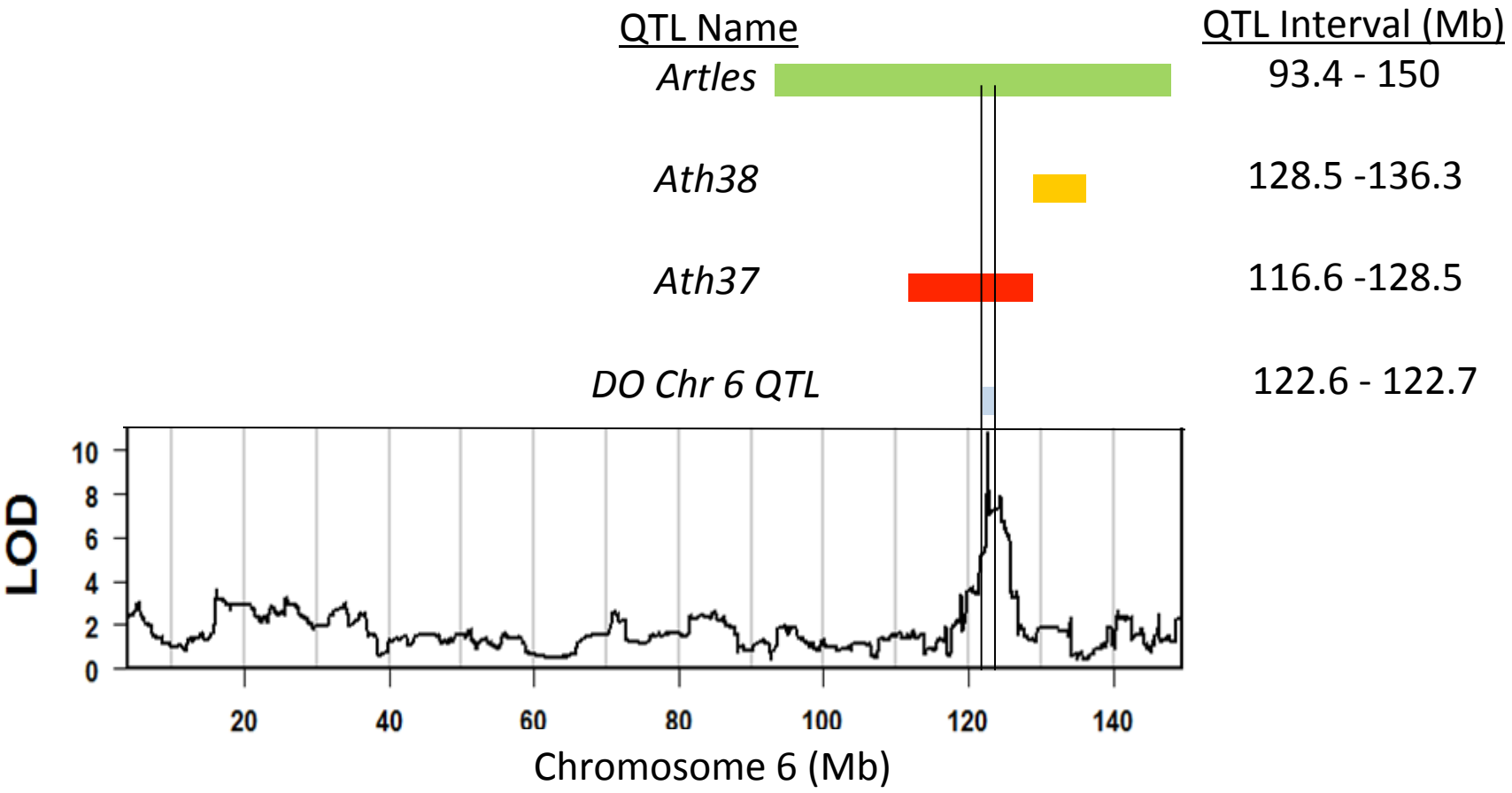
- Mice were on chow diet when received from Jackson Labs.
- Transferred to synthetic diet from 4-6 weeks.
- At 6 weeks of age, half of the mice transferred to a non-atherogenic, low fat diet and half of the mice were transferred to an atherogenic , high fat diet.
- Mice were maintained on these two diets for 18 weeks, until 24 weeks of age.

Diversity Outbred mice: Chromosome 6 associated with atherosclerosis



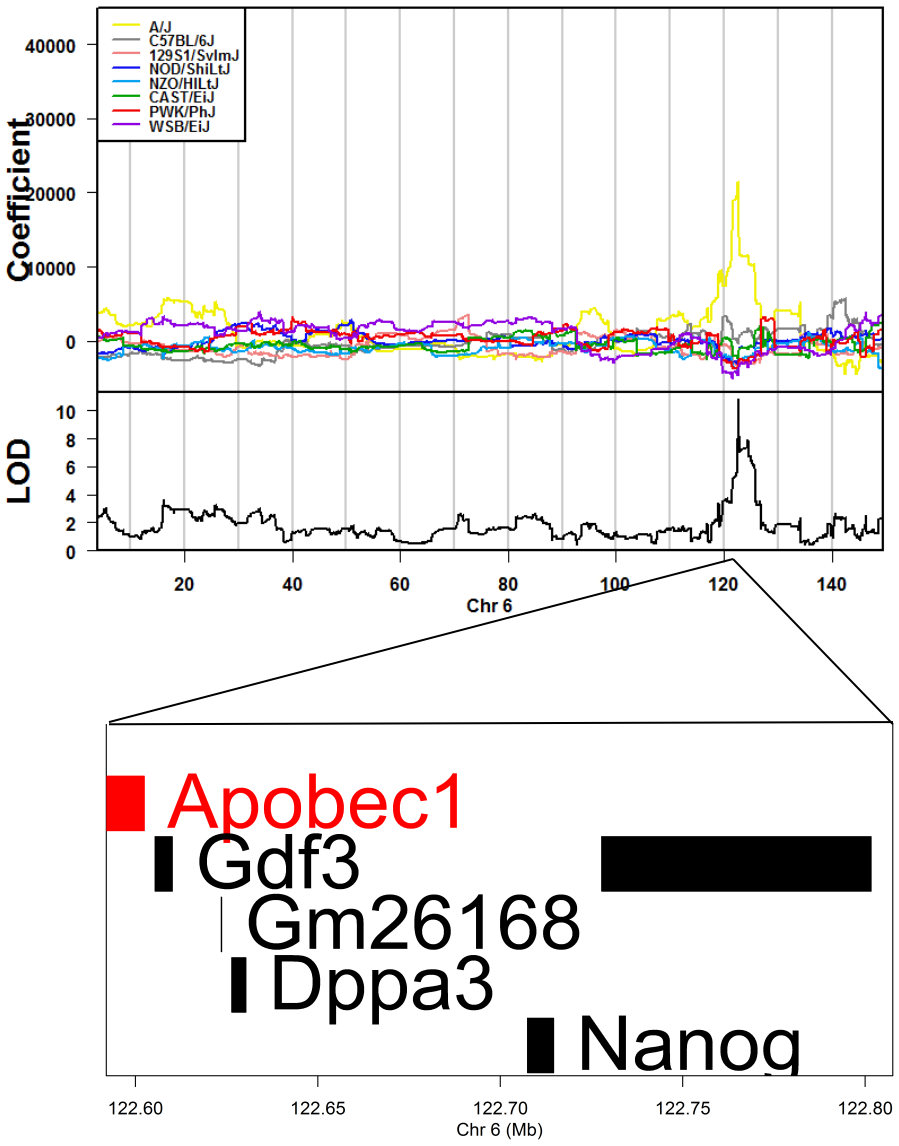
Smallwood 2014

Diversity Outbred mice: Provide high-resolution mapping



We have refined *Ath37* to a 100 kb interval on Chromosome 6.

Diversity Outbred mice: Chromosome 6 associated with atherosclerosis



- The 90,000 kb interval contains 6 genes.
- Likely candidate gene in this region is *Apobec1*.
- Apobec1 is a C → U mRNA editing enzyme.

Integrating Gene Expression-eQTL

Region associated with Lesion size

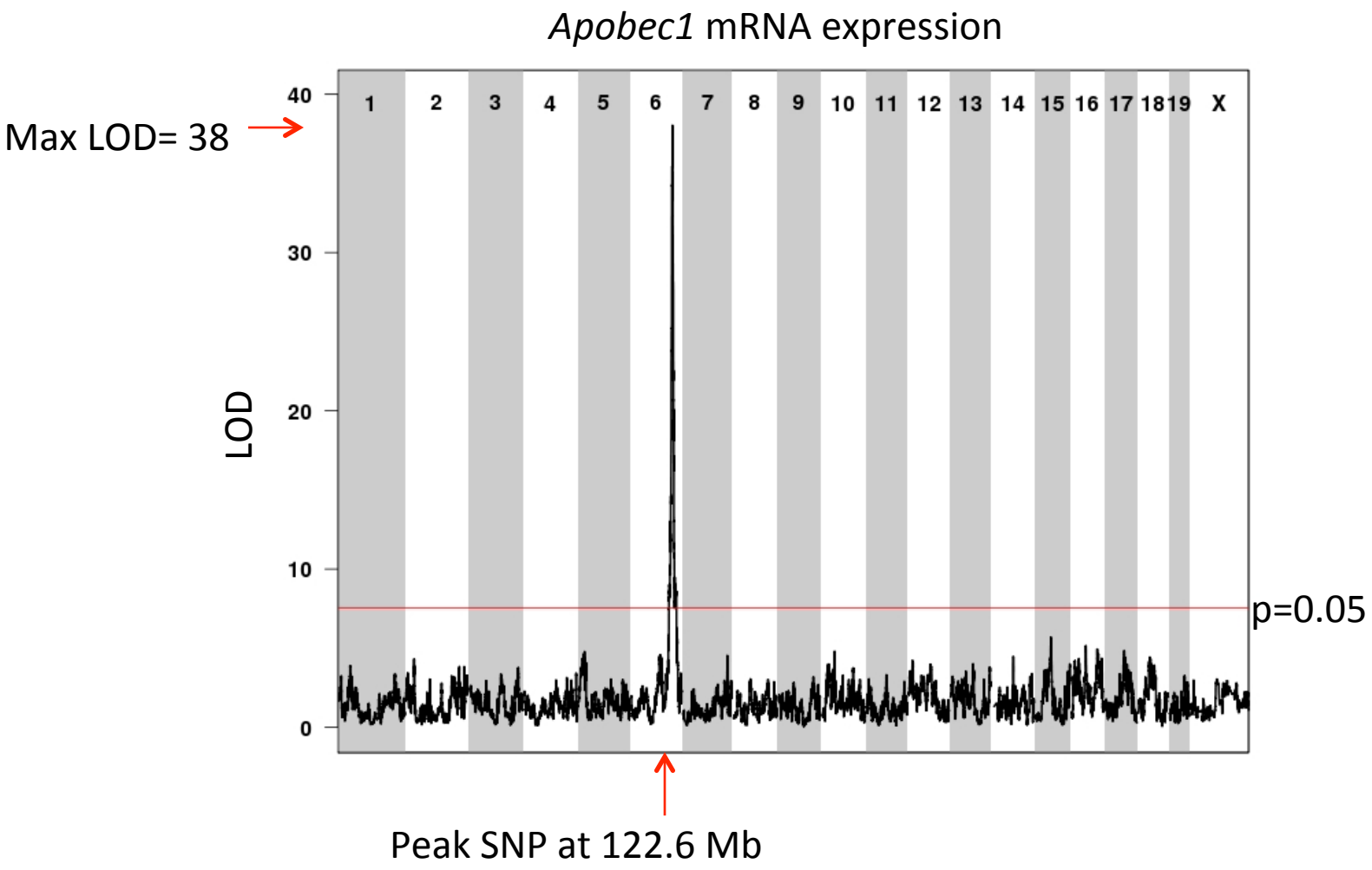
Identify genes whose mRNA's levels is regulated by region

Local eQTL **transcript**

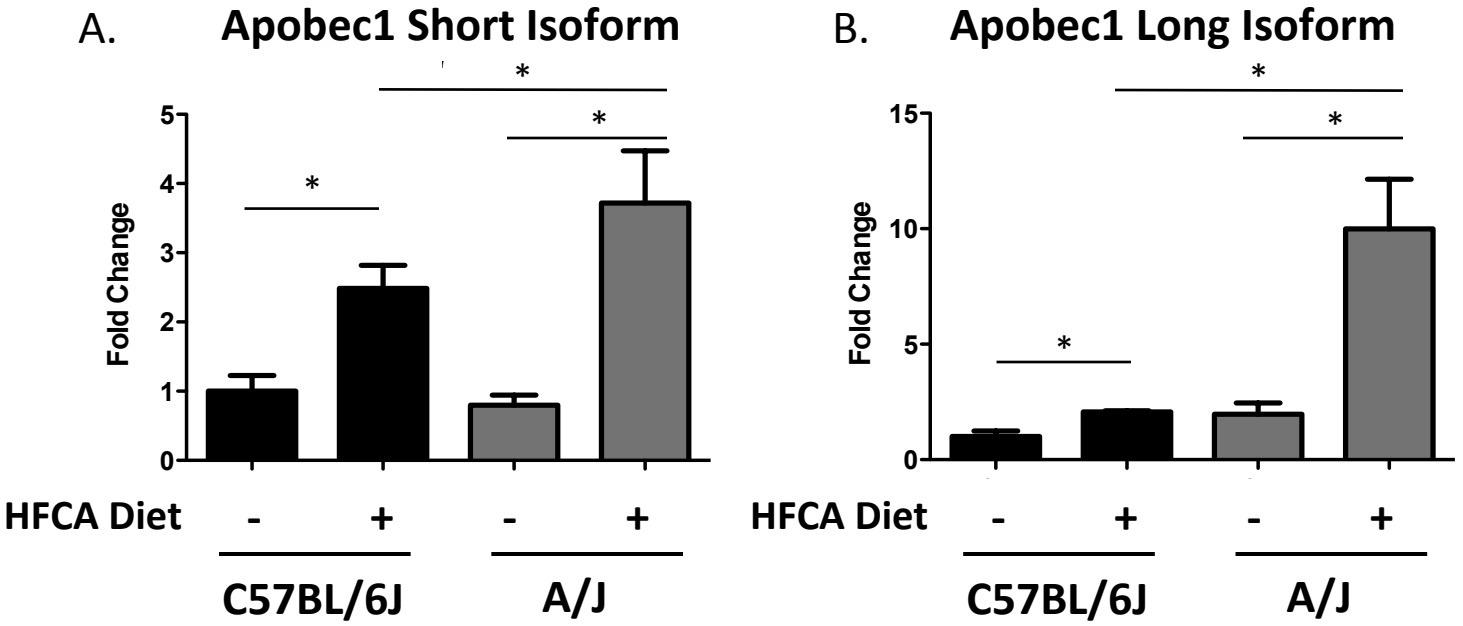
Gene A

QTL co-localizes with the physical location of the gene itself

Integrating Gene Expression-significant local eQTL for *Apobec1*



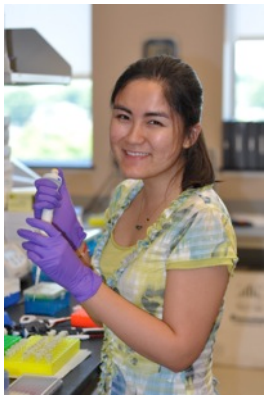
A/J over-expresses the long isoform of *Apobec1* in response to an atherogenic diet



DO mice and atherosclerosis

- We characterized susceptibility of DO mice to diet-induced atherosclerotic lesions
- Refined the previously identified *Ath37* locus on Chromosome 6 to a 100 kb interval containing 6 candidate genes.
- *Apobec1* is a strong candidate gene with known role in lipid biology and atherosclerosis
- Ongoing studies will characterize the A/J allele and how it contributes to atherosclerosis.

Acknowledgements



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