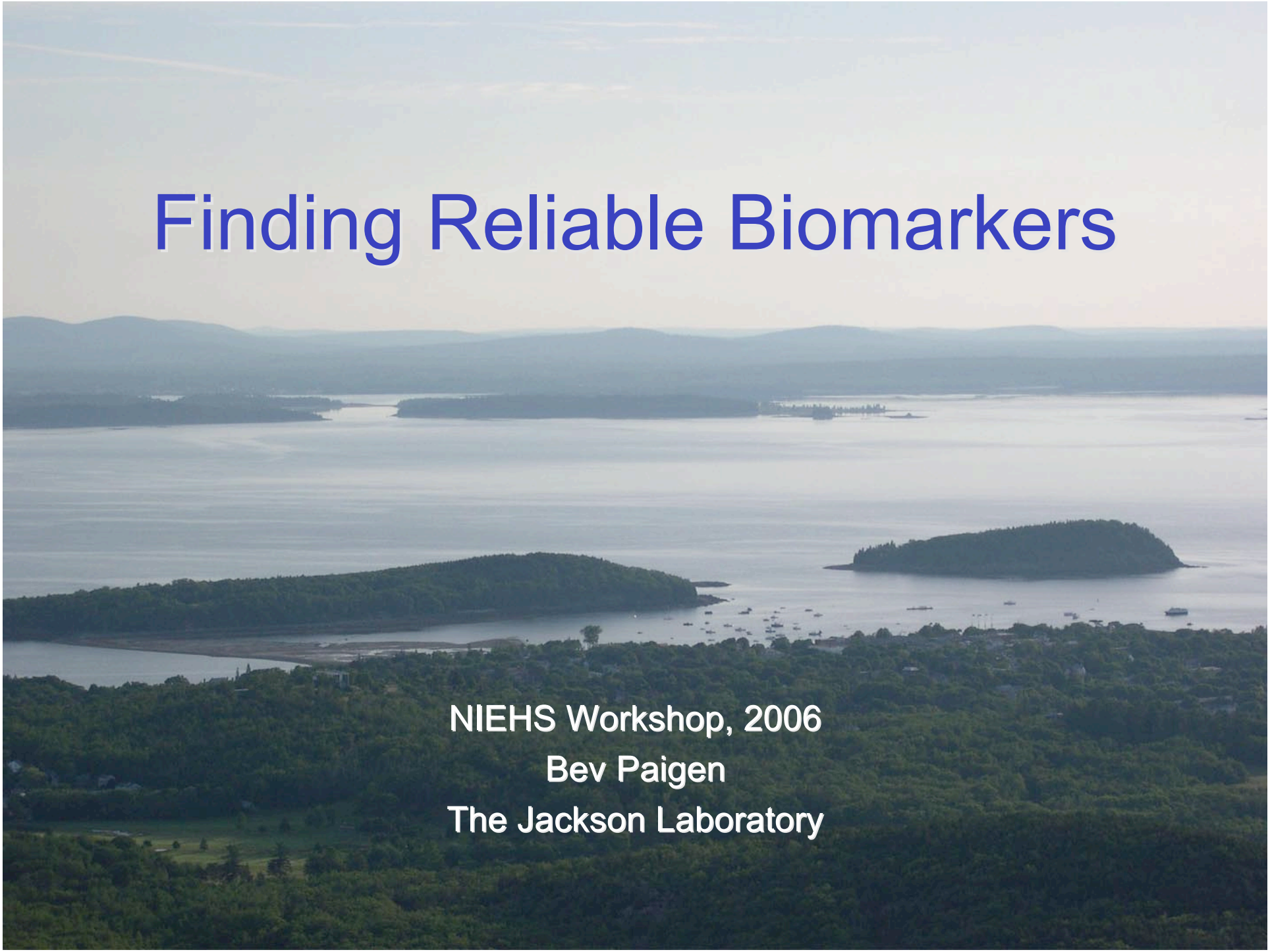


Finding Reliable Biomarkers

NIEHS Workshop, 2006
Bev Paigen
The Jackson Laboratory

This talk is not about biomarkers for lipid and carbohydrate metabolism.

It is about how to find reliable biomarkers for any trait.

Outline

1.Systematic search for biomarkers using inbred strains of mice

2. Determining which biomarkers are most important

3. Can animals be used to find biomarkers for human disease?

Finding Biomarkers

The Phenome Project

1. Measure endpoint (disease) in many inbred strains of mice to mimic genetic diversity of human population.

In general, 40 strains of mice show about the same range of variation as 1000 humans.

Finding Biomarkers

2. Measure as many biomarkers or intermediate phenotypes in these strains as possible.

It is better, but not necessary, if biomarkers are measured on the same mice. With inbred strains, the data can accumulate.

Finding Biomarkers

3. Determine which biomarkers are highly correlated with disease.

Mouse Phenome Database has analysis tools that allow correlations to be done or

Data can be downloaded for analysis.

Mouse Phenome Database

www.jax.org/phenome

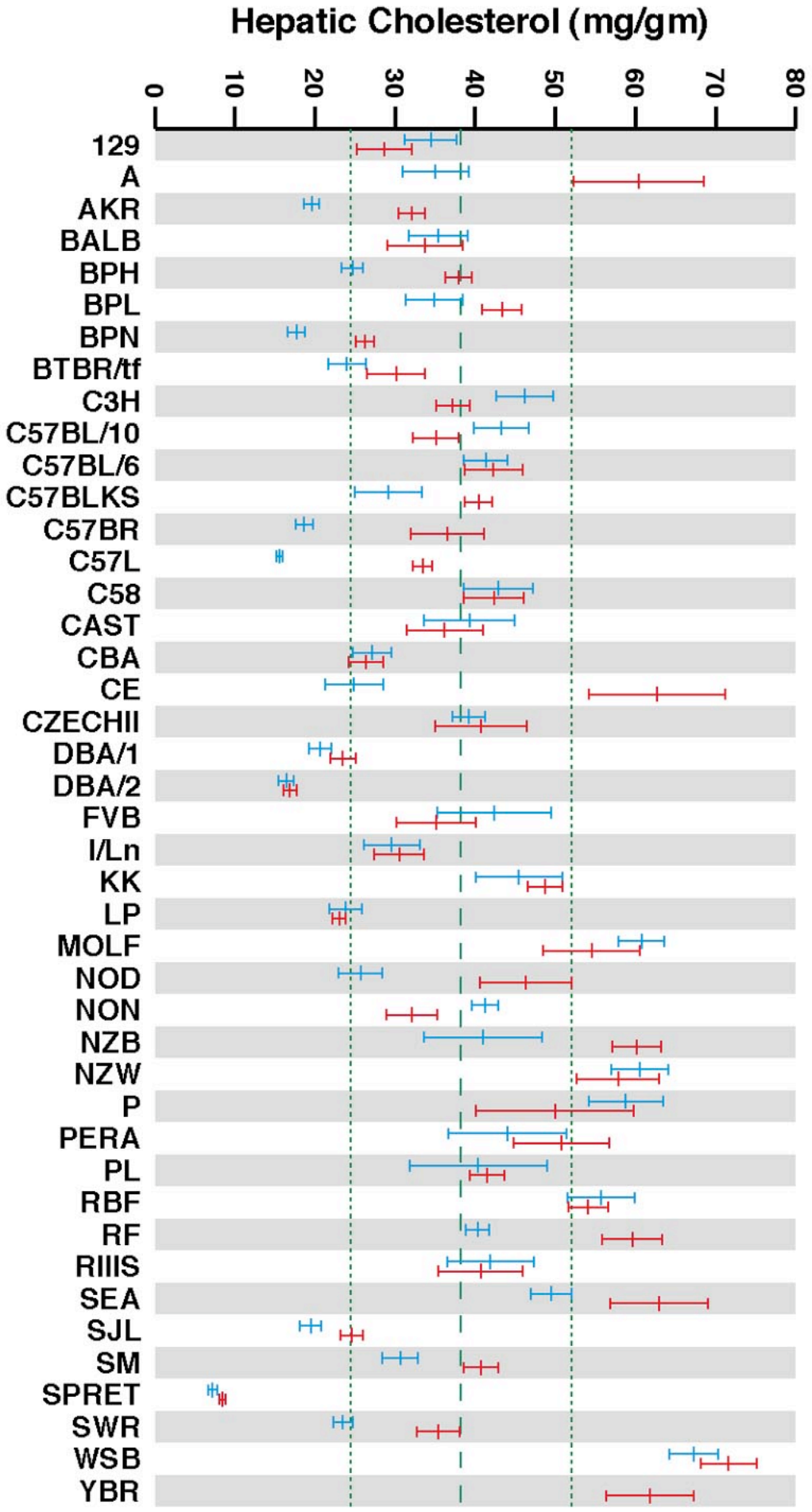
MPD has selected 40 strains for genetic diversity

Measurements made on 10 males and 10 females of each strain at 10 weeks of age

Raw data deposited in MPD

Mouse Phenome Project

- Hemoglobin, other red blood cell parameters
- Complete blood counts
- Blood pressure
- Plasma lipids. Liver lipids
- Lung function, response to methacholine
- Rest/activity patterns
- Atherosclerosis
- Gallstone formation
- Liver response to high fat diet: pathology, enzymes
- Sleep behavior (data not public yet)


Hepatic Cholesterol (mg/gm)
0
10
20
30
40
50
60
70
80
129
A
AKR
BALB
BPH
BPL
BPN
BTBR/tf
C3H
C57BL/10
C57BL/6
C57BLKS
C57BR
C57L
C58
CAST
CBA
CE
CZECHII
DBA/1
DBA/2
FVB
I/Ln
KK
LP
MOLF
NOD
NON
NZB
NZW
P
PERA
PL
RBF
RF
RIIIS
SEA
SJL
SM
SPRET
SWR
WSB
YBR

Little Toxicology Data in MPD

Major environmental stressor is high fat diet.

Would be very desirable to have data on other environmental stressors and the responses in multiple mouse strains.

Bonus

Because dense genotyping with SNPs has been done on these inbred strains (much of it thanks to NIEHS resequencing effort), a strain survey of disease or biomarkers (or any phenotype) can be used for QTL analysis to find genes that determine the trait.

Haplotype Association Mapping

- Search for an association of haplotype with phenotype over multiple strains
- Recent attempt using 25 strains and 12,000 SNPs showed reasonable agreement between predicted QTLs and QTLs found in crosses- Pletcher Plos Biology 2004
- Many improvements in method since the Pletcher paper

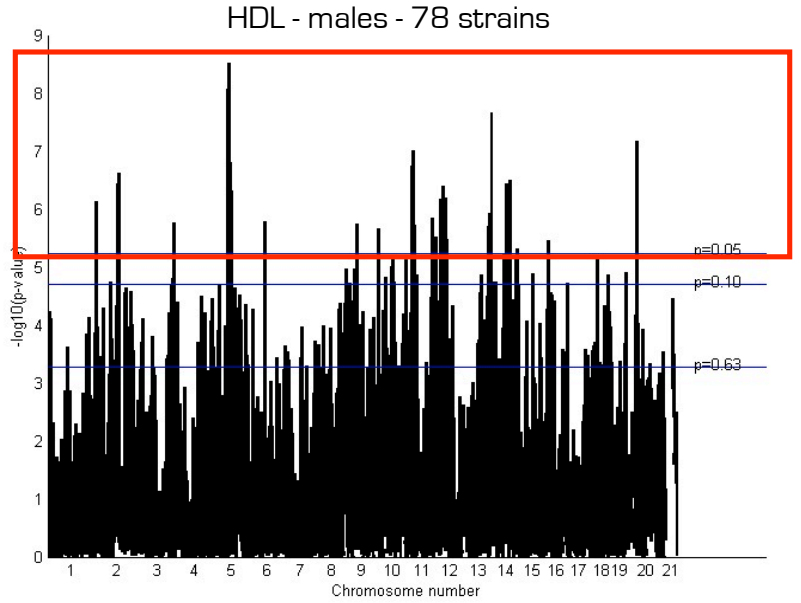
Haplotype Association Mapping of QTLs

Trait is HDL cholesterol

- Used 78 strains
- Found at least 15 significant peaks
- Tested these against crosses
- Peaks were real

or were in LD with

real peaks



Which Biomarkers are Important?

Use *structural equation modeling* and all biomarkers correlated with disease to determine relationships.

Process applied to this problem by Li and Churchill and described in Li et al PLoS Genetics 7:114, 2006

Possible Relationships

Biomarker could be upstream or causal to disease.

Biomarker could be downstream-caused by disease.

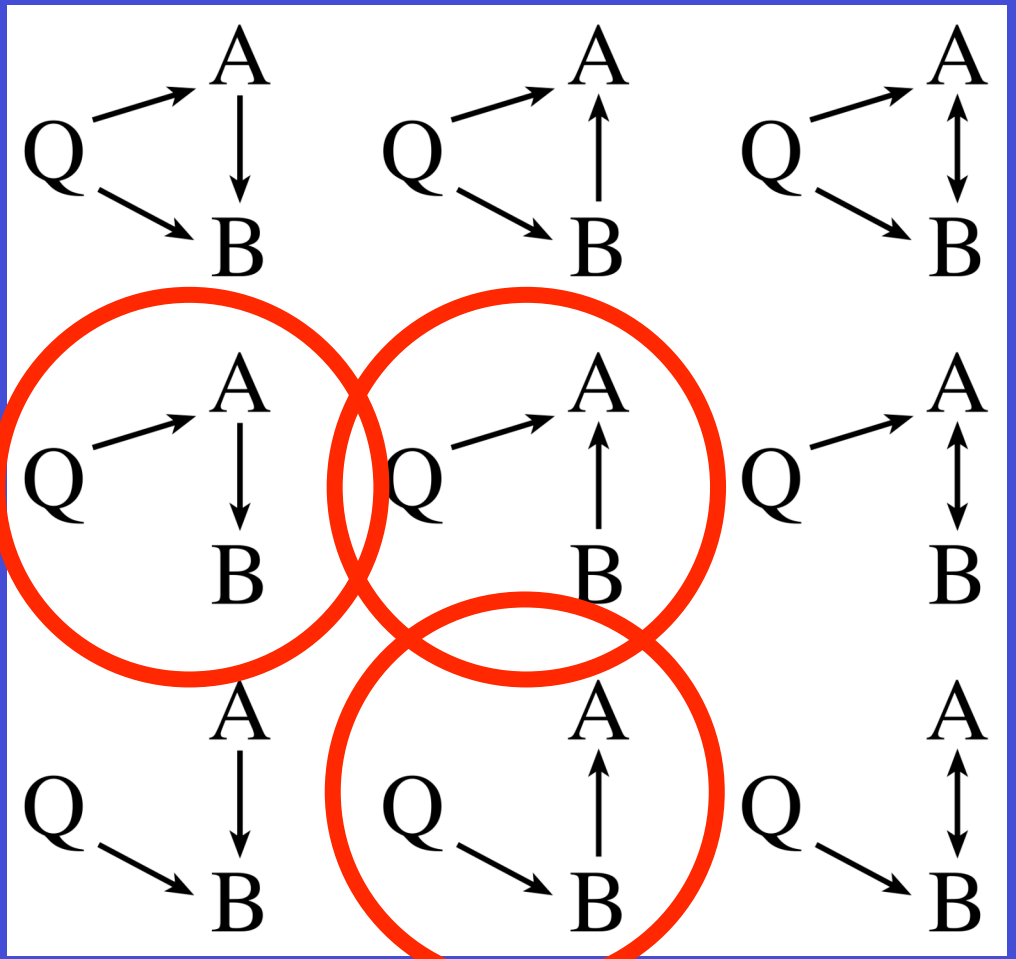
Two biomarkers could independently affect disease.

One biomarker could affect the second, which affects the disease.

Biomarkers could interact with each other.

and many variations

Relationship between a QTL and two correlated traits

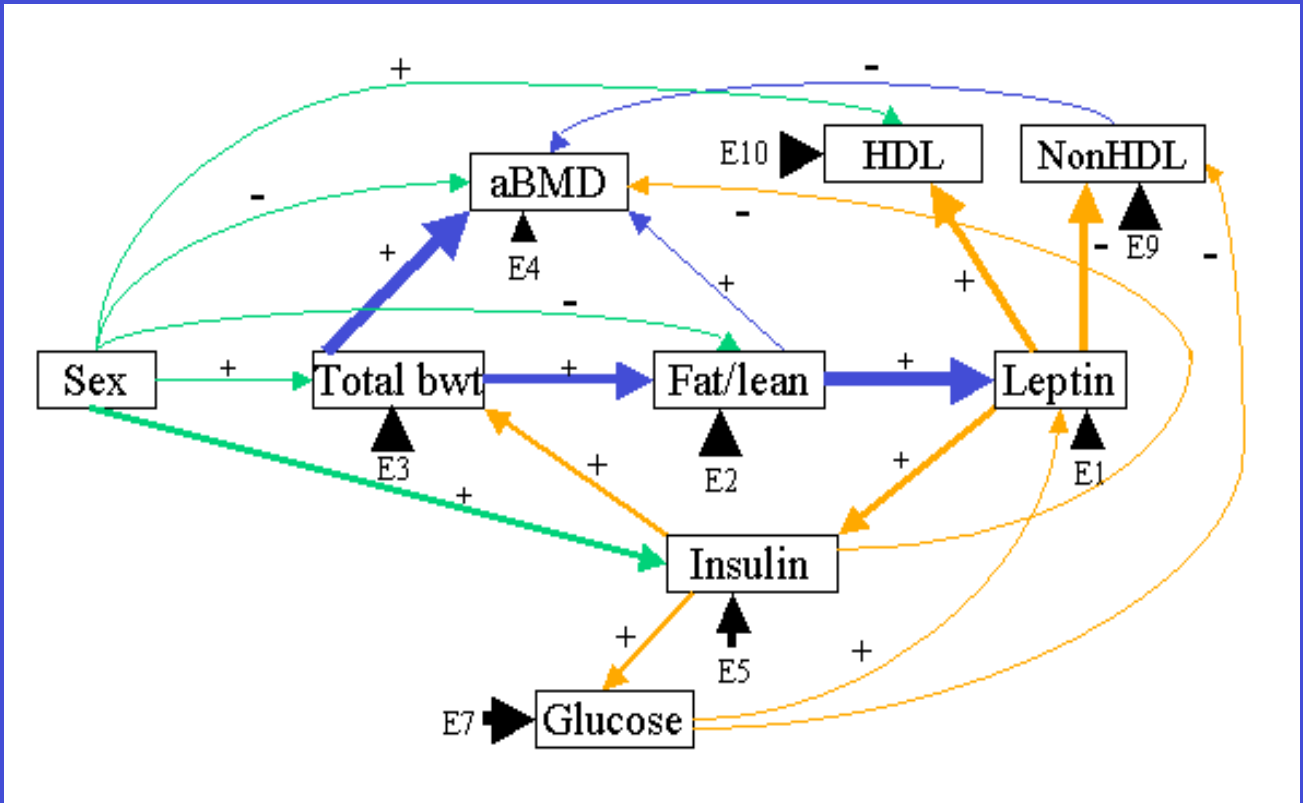


Using Modeling on Strain Data

Structural modeling applied to strain survey data of lean body weight, % fat, bone density, leptin, plasma lipids, glucose, insulin.

Note: previously observed that fat mice usually had high HDL levels, which is different from humans.

Mouse Inbred Strain Survey



Structural Modeling for Chr 12

A QTL for HDL cholesterol on Chr 12 had 3 peaks.

Microarray data pointed to 3 candidate genes with differential expression- all correlated with each other and with HDL. Structural modeling was used to test relationships.

Summary (so far)

1. A survey of disease endpoint and multiple biomarkers across a genetically diverse set of inbred strains shows which biomarkers are correlated with disease.
2. Structural equation modeling of correlated biomarkers reveals the relationships.
3. These data can be used to select the biomarkers that will provide the critical information (depending on what the question is).

Can Animals be Used to Find Biomarkers for Human Disease?

Question has not been answered for biomarkers. (to the best of my knowledge)

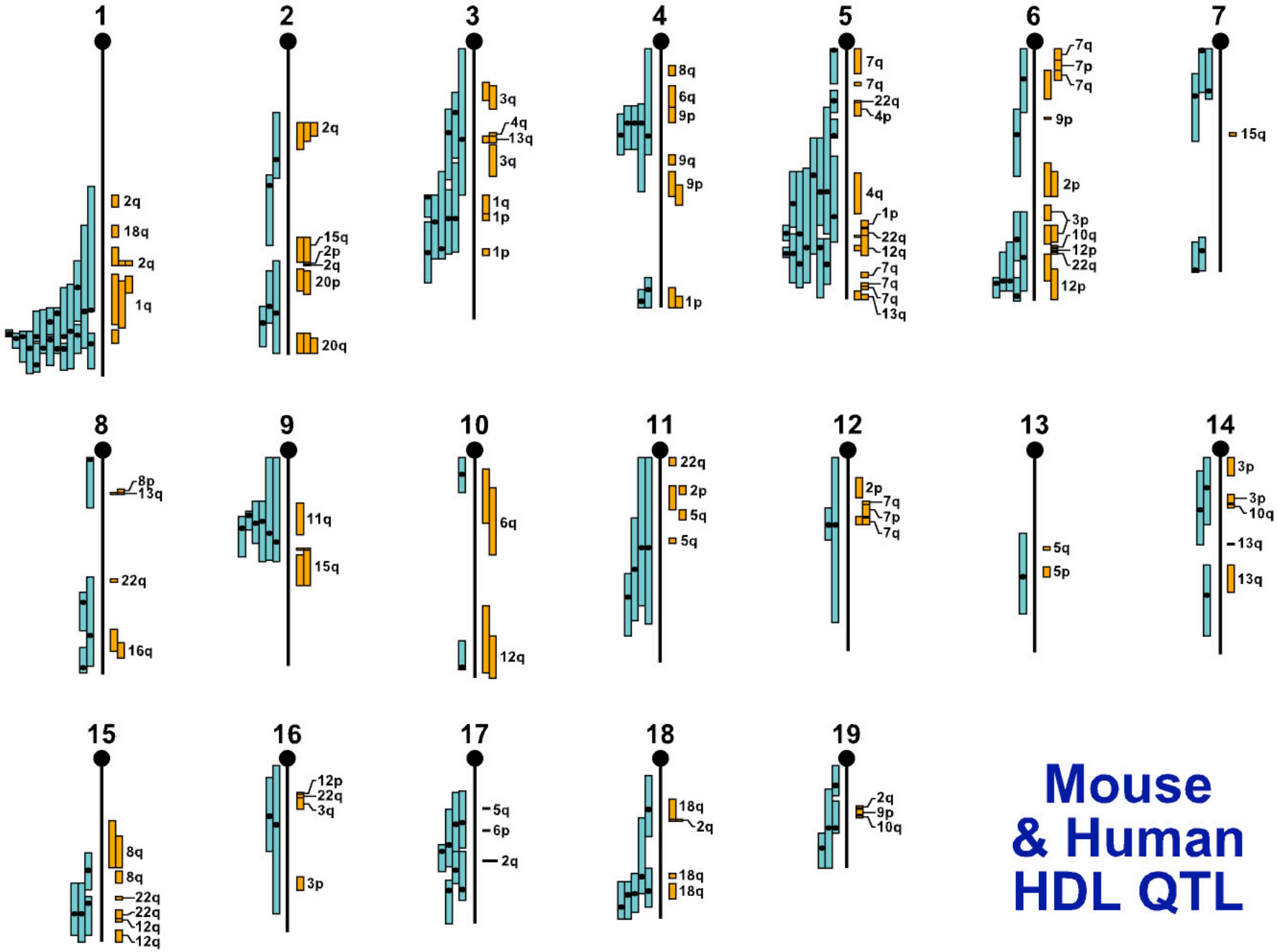
Question has been answered for whether animals can be used to find genetic determinants of disease.

Can Animals be Used to Find Biomarkers for Human Disease?

Chose traits with quantitative trait loci (QTL) mapped in both mice and humans: *hypertension*, *bone density*, *plasma lipids*, *asthma*, *inflammatory bowel disease, kidney disease.*

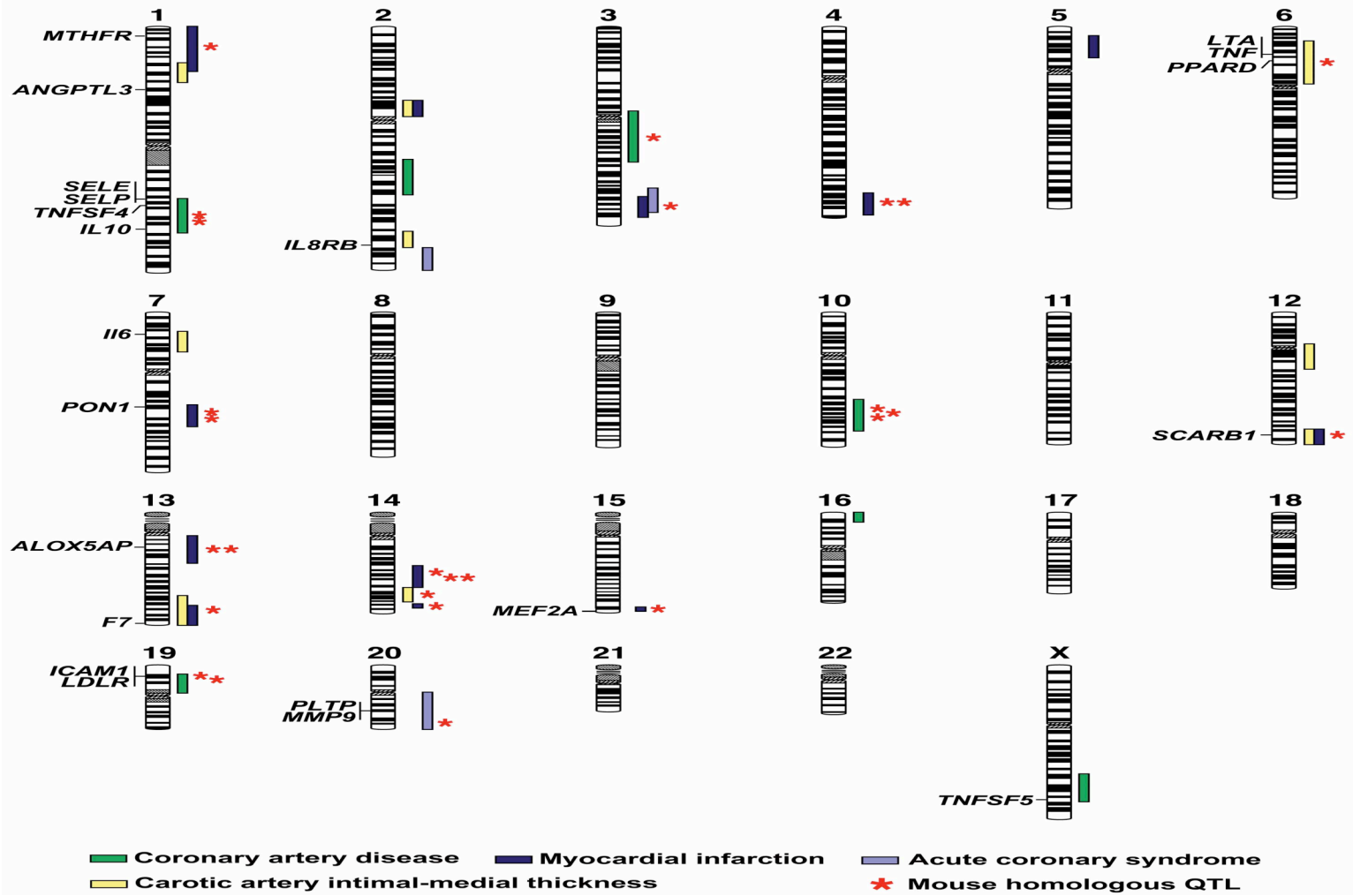
Searched literature, counted QTL significant in one study, or suggestive in one and replicated in at least one other study.

Placed mouse QTL on map and human QTL in homologous regions.



Mouse & Human HDL QTL

Human Atherosclerosis QTLs



Conclusions

All traits showed a high degree of concordance between human and mouse QTL.

For blood pressure, rat also showed a high degree of concordance.

Why Concordance?

- QTL represent a finite set of key regulatory genes.
  (only some genes in pathway are key regulatory)
- Only mutations in these key regulatory genes lead to a change in the phenotype.
- These regulatory systems are evolutionarily conserved between mouse and human.

Why Concordance?

If there is evolutionary conservation of

- Genome- gene sequence and arrangement
- Structure-anatomy
- Function-physiology and molecular circuits
- Regulatory systems- QTLs

It is likely that biomarkers may also be shared between human and mouse.

An Example- Hepatitis, liver fibrosis and CCl_4 -Hillebrandt Nat Genet 2005

- Strains of mice were treated with CCl_4 and screened for liver fibrosis.
- Cross between susceptible and resistant strains mapped QTL, haplotype mapping identified complement C5 as the gene.
- C5 loss confers resistance to other inducers of liver fibrosis including hepatitis, treatment with C5 antibody also works.
- Polymorphisms of C5 associated with fibrosis in humans.

Acknowledgements

Keith DiPetrillo
Xiaosong Wang
Ioannis Stylianou
Sarah Burgess
Zhiguang Su

Gary Churchill
Sharon Tsaih
Renhua Li