

# Using human GWAS data to interrogate complex traits in an outbred mouse population



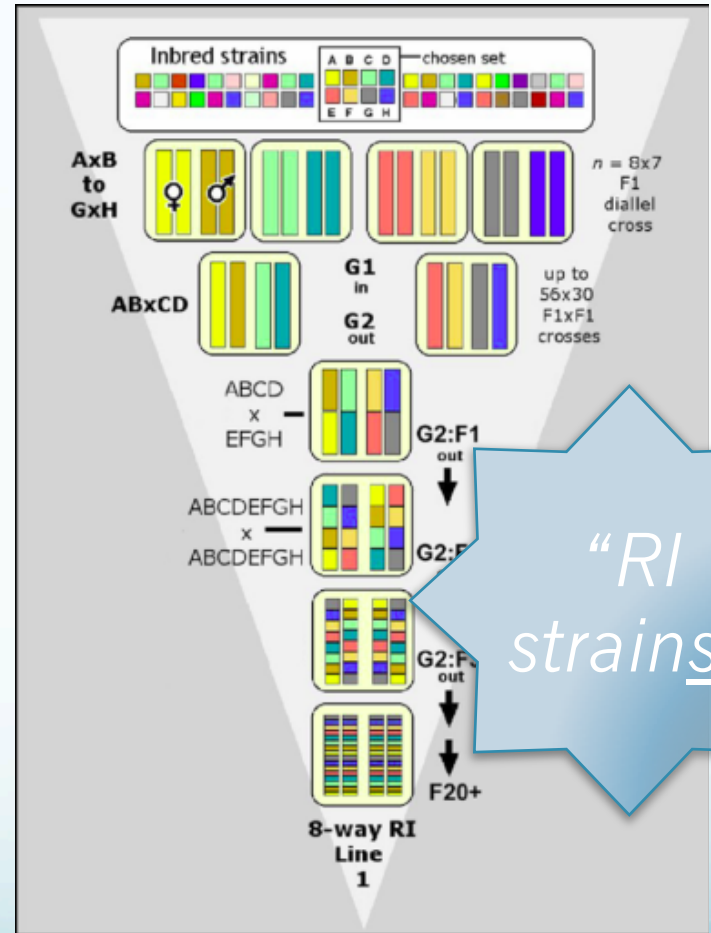
*Karen L. Svenson*

*Research Scientist*

*The Jackson Laboratory*

*GWAS Catalog Webinar 18 July 2013*

# The Collaborative Cross (“CC”)



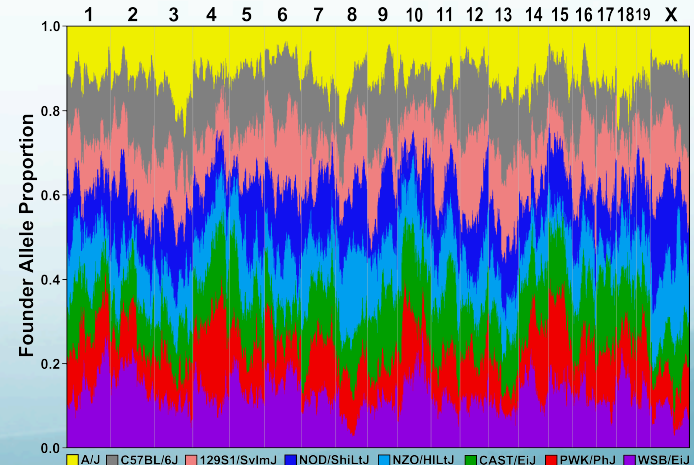
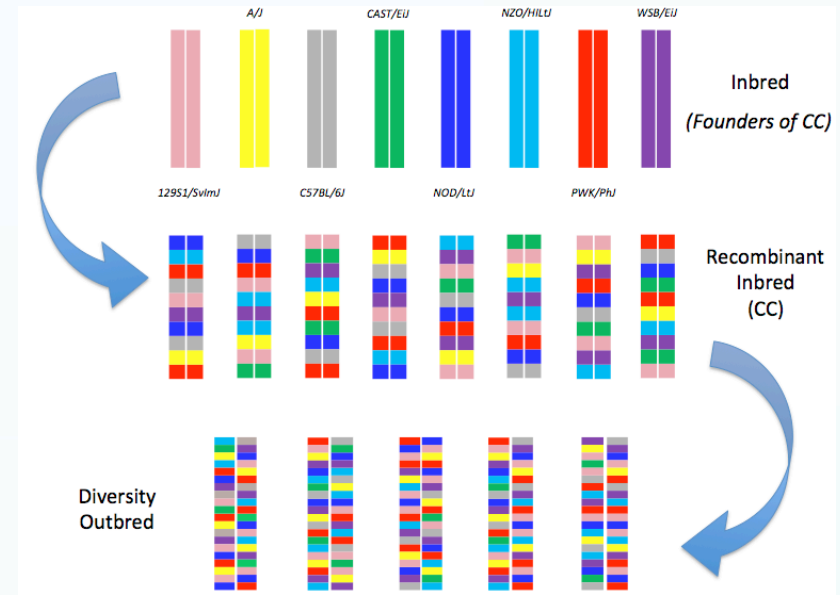
<http://csbio.unc.edu/CCstatus/index.py>

“Funnel” breeding design ensures balanced contributions of sex chromosomes and mitochondria

# The DO

## Diversity Outbred mouse population

- Derived from partially inbred lines of the CC (F4-12)
  - Share 8 founder inbred strains
- Randomized breeding scheme
  - 175 breeding pairs contribute 1 female and 1 male offspring to the next generation
- 90% heterozygosity at G5
  - <10Mb recombination segments
- Currently breeding G12
  - JAX stock No. 9376
- MUGA 7.5K SNP genotyping chip
  - Mega-MUGA 70K *NOW AVAILABLE*



# Study Design

## Phenotyping

23-week protocol

- Chow OR HF diet from wean age
- Non-invasive, high throughput screens
- Includes repeat measurements  
(track change with diet, age)

## Genotyping

- MUGA 7.5K SNP array
- 550 DO (300ch; 250hf)
- G4-8

Test	Age (weeks)																							
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
<i>Begin chow OR HF diet</i>																								
Weight																								
Plasma chemistries w/fast 1																								
Whole blood analysis 1																								
Urinalysis 1																								
Body composition 1																								
EKG																								
Plasma chemistries: nonfasted																								
Plasma chemistries w/fast 2																								
Urinalysis 2																								
Body composition 2																								
Whole blood analysis 2																								
ipGTT 20 select females																								
ipGTT 20 select males																								
Necropsy (organ wts, harvest)																								

103 phenotypic parameters

# QTL Analysis

	Phenotype	LOD	p value	Chr
1	CHOL2	10.9	0	1
2	HDL2	11.0	0	1
3	CHCM1	12.8	0	7
4	HDW1	11.3	0	9
5	CHCM2	13.6	0	7
6	HDW2	12.7	0	9
7	CHCM2	9.8	0.001	9
8	HDL1	8.7	0.003	1
9	RBC1	7.8	0.013	8
10	CHOL1	7.4	0.02	1
11	cHGB2	7.4	0.022	7
12	MCHC1	7.4	0.029	7
13	NEUT2	7.4	0.03	8



Cholesterol (HDL chol)  
Test 1, Test 2  
*ApoAII*



Whole blood (Red cell related)  
Test 1, Test 2  
*Hbb complex, transferrin*

Where are the QTL for **my**  
favorite traits?????

# Metabolic Syndrome in the DO: Case Studies

- Where are the QTL for

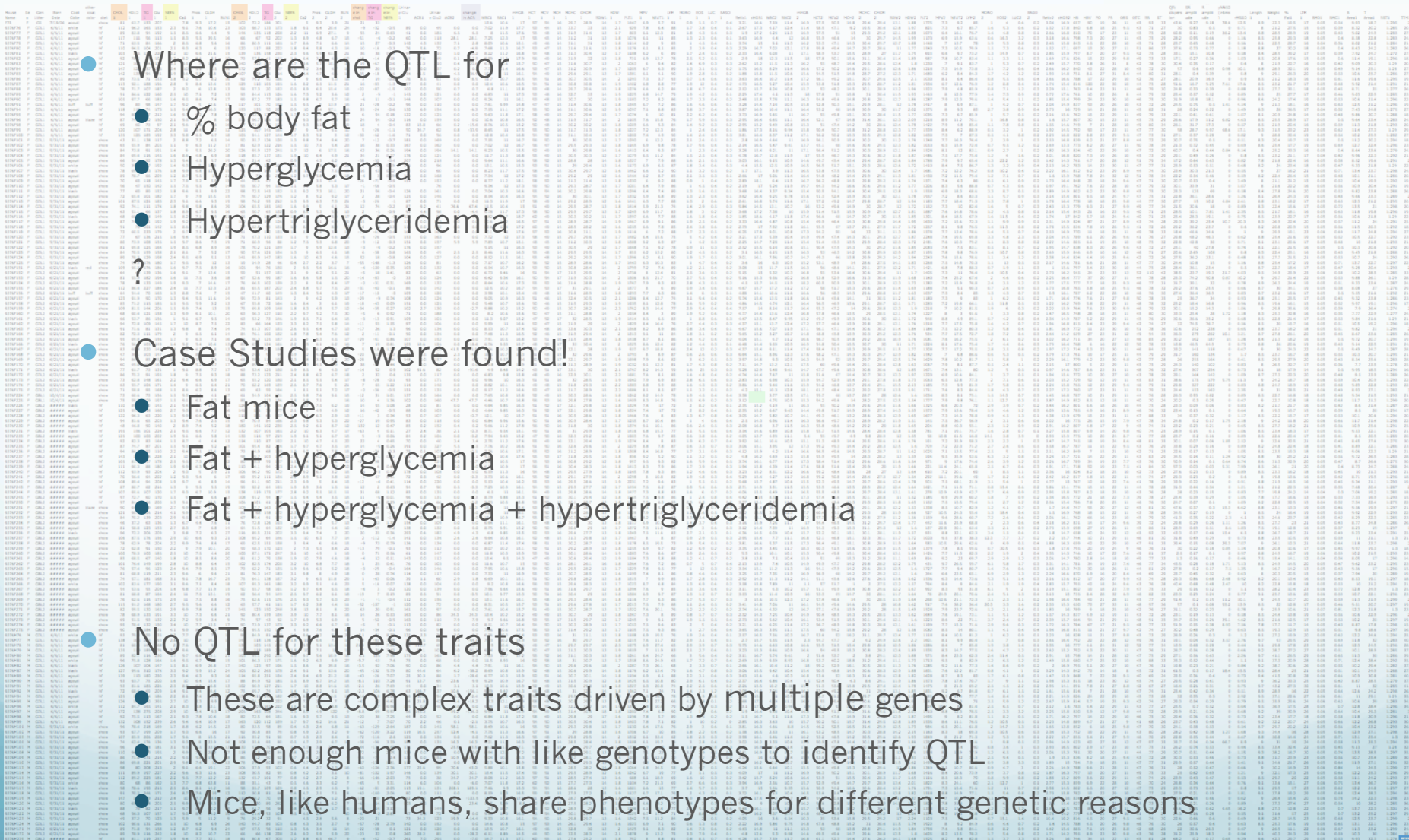
- % body fat
- Hyperglycemia
- Hypertriglyceridemia
- ?

- Case Studies were found!

- Fat mice
- Fat + hyperglycemia
- Fat + hyperglycemia + hypertriglyceridemia

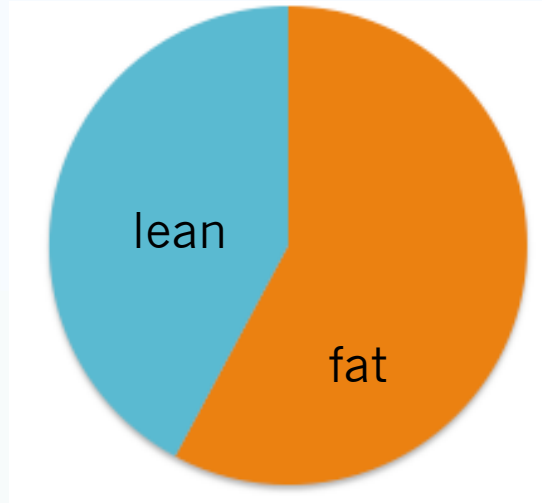
- No QTL for these traits

- These are complex traits driven by multiple genes
- Not enough mice with like genotypes to identify QTL
- Mice, like humans, share phenotypes for different genetic reasons



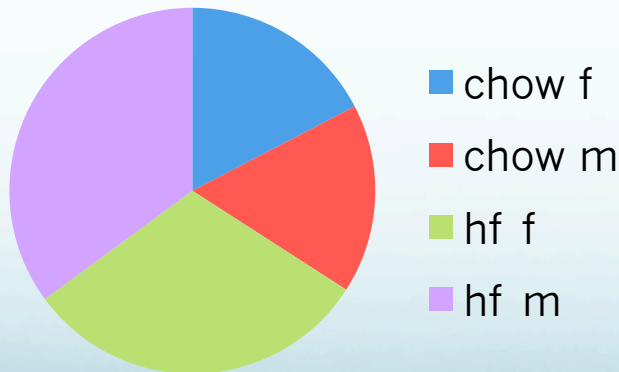
# Obesity in the D0

*All Mice*



*58% got fat*

*Fat Mice*



Chow:

36% females got fat

37% males got fat

High Fat Diet:

75% females got fat

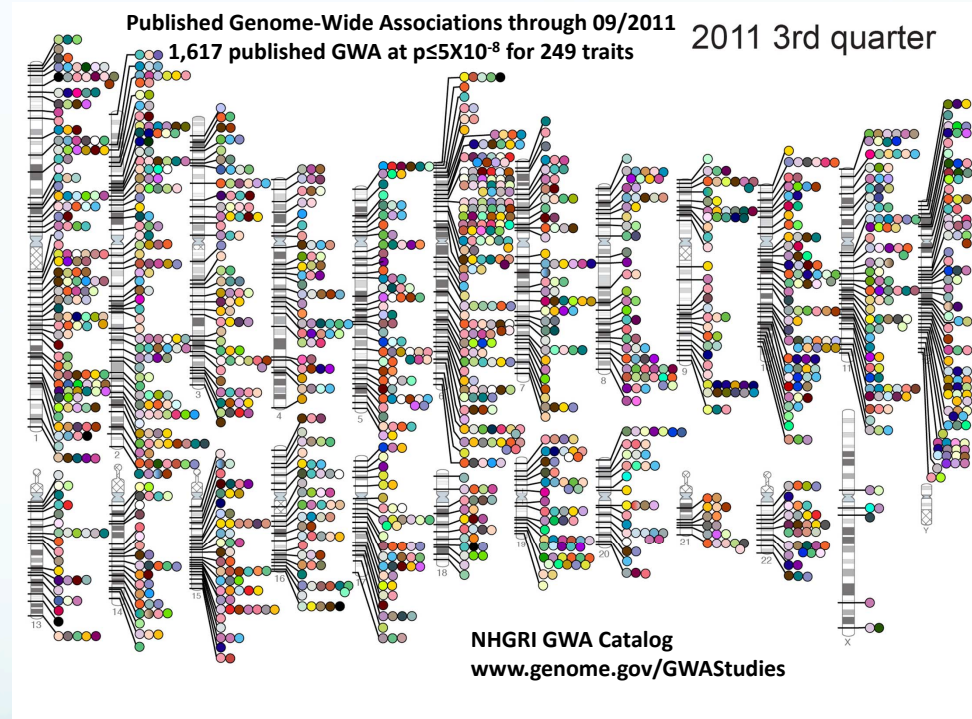
91% males got fat



# New Approach



- Select only fat mice
- Survey Human GWAS for plausible loci related to MetSyn
- Find talented summer student
- Create effect plots and QTL scans in R; look for allele bias at select GWAS loci



Nicole Savignac, NCSSM, GA Tech



# Choosing GWAS Hits

- >1200 significant hits in 1607 studies associated with
  - Glucose/T2D
  - Obesity (BW, BMI, waist circumference, WtH ratio)
  - Triglycerides
- = 678 genes
  - Rank by number of times found (1-30)
  - Find homologous mouse locus\*\*\*
- Start with 10
  - Found 11-30 times

	A	B	C	D
	Mouse Gene	Number of Studies	Mouse Chr	Mouse bp
1	Lpl	30	8	71404390-71431347
2	Fto	27	8	93837431-94192338
3	Zfp259	25	9	46081147-46090726
4	Apob	24	12	7984454-8023641
5	Apoa5	23	9	46076716-46080002
6	Gckr	22	5	31599954-31629673
7	Herpud1	21	8	96910338-96919277
8	Apoa1	19	9	46036663-46038549
9	Apoc3	18	9	46041016-46043719
10	Apoa4	17	9	46048779-46051542
11	Lipc	17	9	70645935-70782615
12	Tcf7l2	17	19	55816310-56008144
13	Apoe	16	7	20281458-20284515
14	Fads1	16	19	10257378-10271360
15	Cdkal1	15	13	29283615-29947543
16	Bud13	14	9	46091091-46106866
17	Fam84b	14	15	60650551-60656635
18	Mtnr1b	14	9	15667058-15679000
19	Trib1	14	15	59479905-59488105
20	Apoc1	12	7	20274833-20278007
21	Celsr2	12	3	108193769-108218470
22	Fads2	12	19	10138654-10175993
23	Cilp2	11	8	72404268-72411586
24	Dock7	11	4	98603366-98787551
25	Ldlr	11	9	21528038-21554360
26	Mc4r	11	18	67017369-67020126
27	Mixipl	11	5	135582761-135614252
28	Sic30a8	11	15	52127108-52167353
29	Abca1	10	4	53043659-53172767
30	Acaa2	10	18	74938866-74965861
31	Cdkn2b	10	4	88952198-88956941
32	Fads3	10	19	10116038-10134161
33	G6pc2	10	2	69049130-69065898
34	Hmgcr	10	13	97418922-97440891
35	Lipg	10	18	75098976-75120917
36	Ncan	10	8	72616984-72644772
37	Apoc2	9	7	20256933-20266772

# Effect Plots

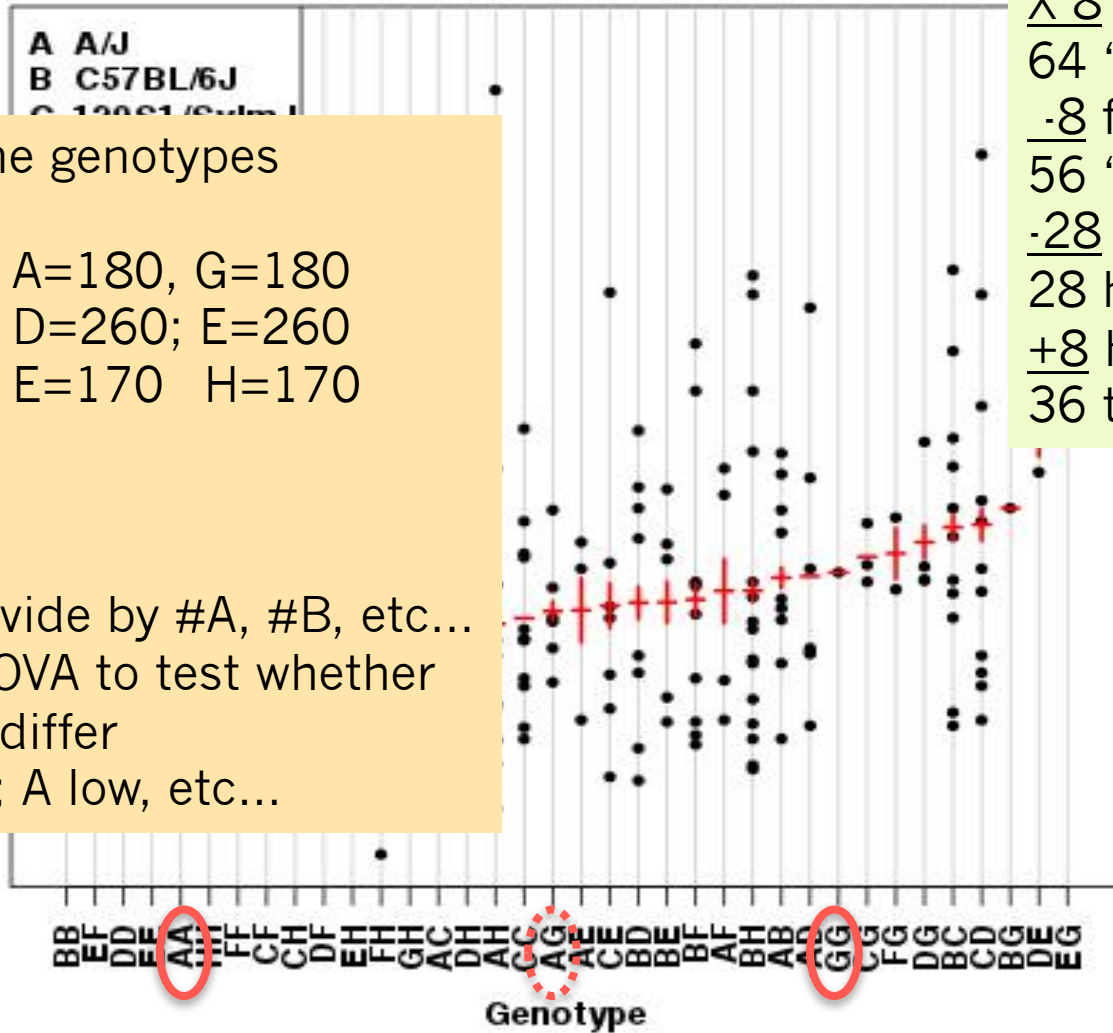
G6pc2

8 founder strains  
 $\times$  8 founder strains  
 64 "F1" crosses  
 $\underline{-8}$  founder strains  
 56 "diallels"  
 $\underline{-28}$  reciprocals  
 28 heterozygotes  
 $\underline{+8}$  homozygotes  
 36 total genotypes

Weighting the genotypes

AG = 180      A=180, G=180  
 DE = 260      D=260; E=260  
 EH = 170      E=170    H=170

Add all As  
 Add all Bs  
 Add all Cs  
 Etc... and divide by #A, #B, etc...  
 Perform ANOVA to test whether  
 Groups A-H differ  
     G high; A low, etc...



Genotypes  
 [TG]

ANOVAs

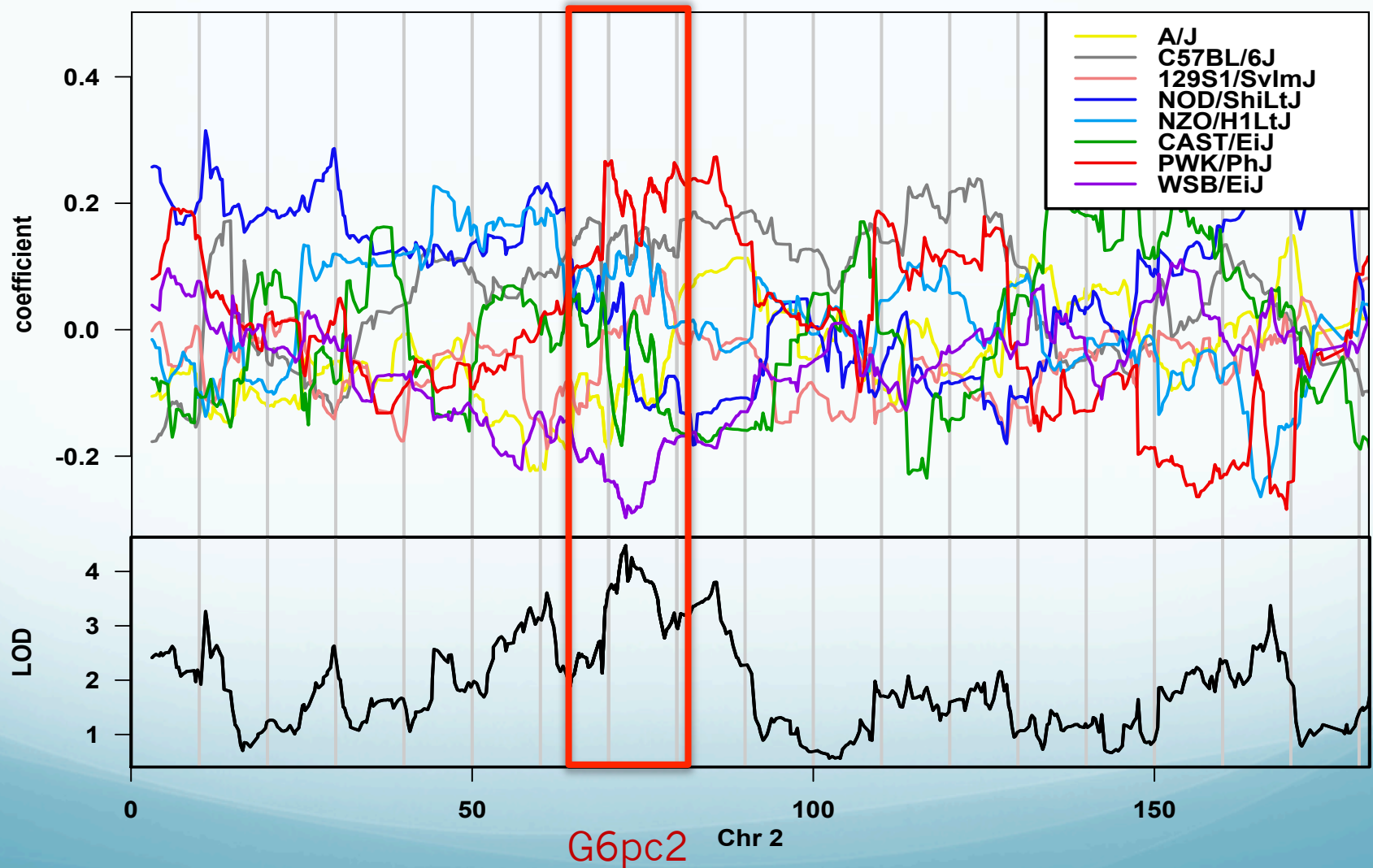
# Effect Plots - Results

Gene	Phenotype	ANOVA Value in OB Mice	ANOVA Value in non-OB Mice
Apoa1	Triglycerides	0.0103	0.5961
Bud13	Triglycerides	0.0142	0.5751
G6pc2	Glucose	0.0036	0.6298
G6pc2	Triglycerides	<0.0001	0.3257
Tcf7l2	Triglycerides	0.0415	0.5666

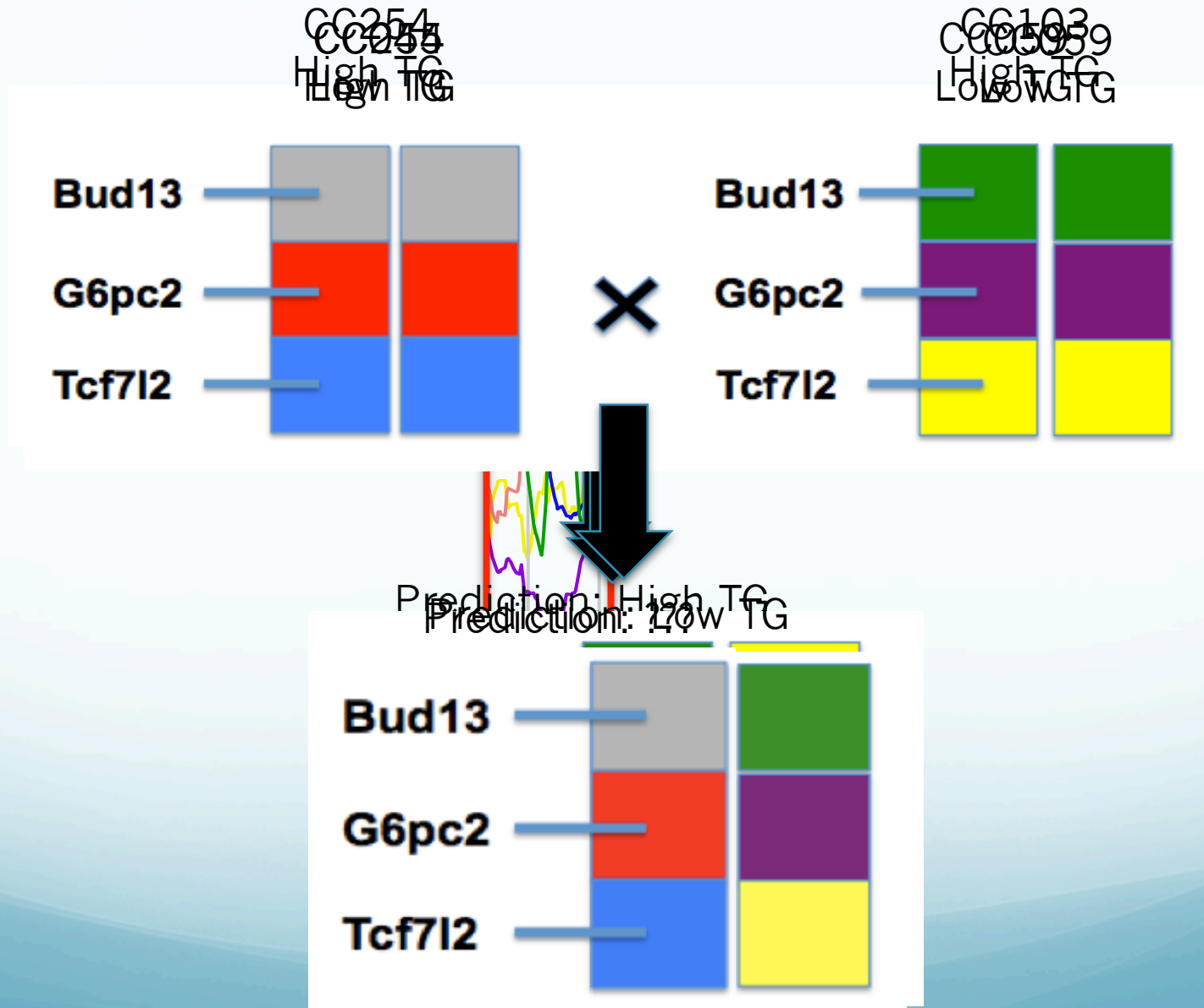
- 4/(7)10 GWAS loci were found associated with MetSyn traits, not previously revealed by QTL analyses or summary statistics of all data.
- Subgrouping by phenotype helps reveal what is observed in ‘case studies’; there is more information to be found in these data using alternate approaches

# Founder effect plot

TG2: Obese mice



# Predictive Genetics: CC RIX



# Project status



Presentation at IMGC October 2012

Terry Meehan, EBI resources/HP

Is Nicole available summer 2013?

Karen/Nicole @ EBI July 1, 2013

Nicole on EBI R cloud

Has written code to analyze all 678 genes for  
>100 phenotypes (+100 mice; now 650)

Working to automate identification of  
orthologous mouse loci in the GWAS catalog

Predictions/visualization strategies ongoing

# Conclusions

- The DO are complex and require new computational approaches to finding both main effect and interacting loci underlying phenotypes; “case studies” are important but lack power for use in current QTL analysis strategies
- The DO can be used to mimic human GWAS populations and may serve to validate GWAS hits, thereby identifying new mouse models of human disease. (human/mus/human...)
- New analyses developed for the DO can lead us to predictive genomic approaches towards individualized medical analysis and treatments/interventions in humans



# Production, Analysis of the DO



Marge Strobel

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JAX Mice and Services™



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...more