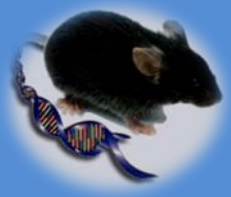
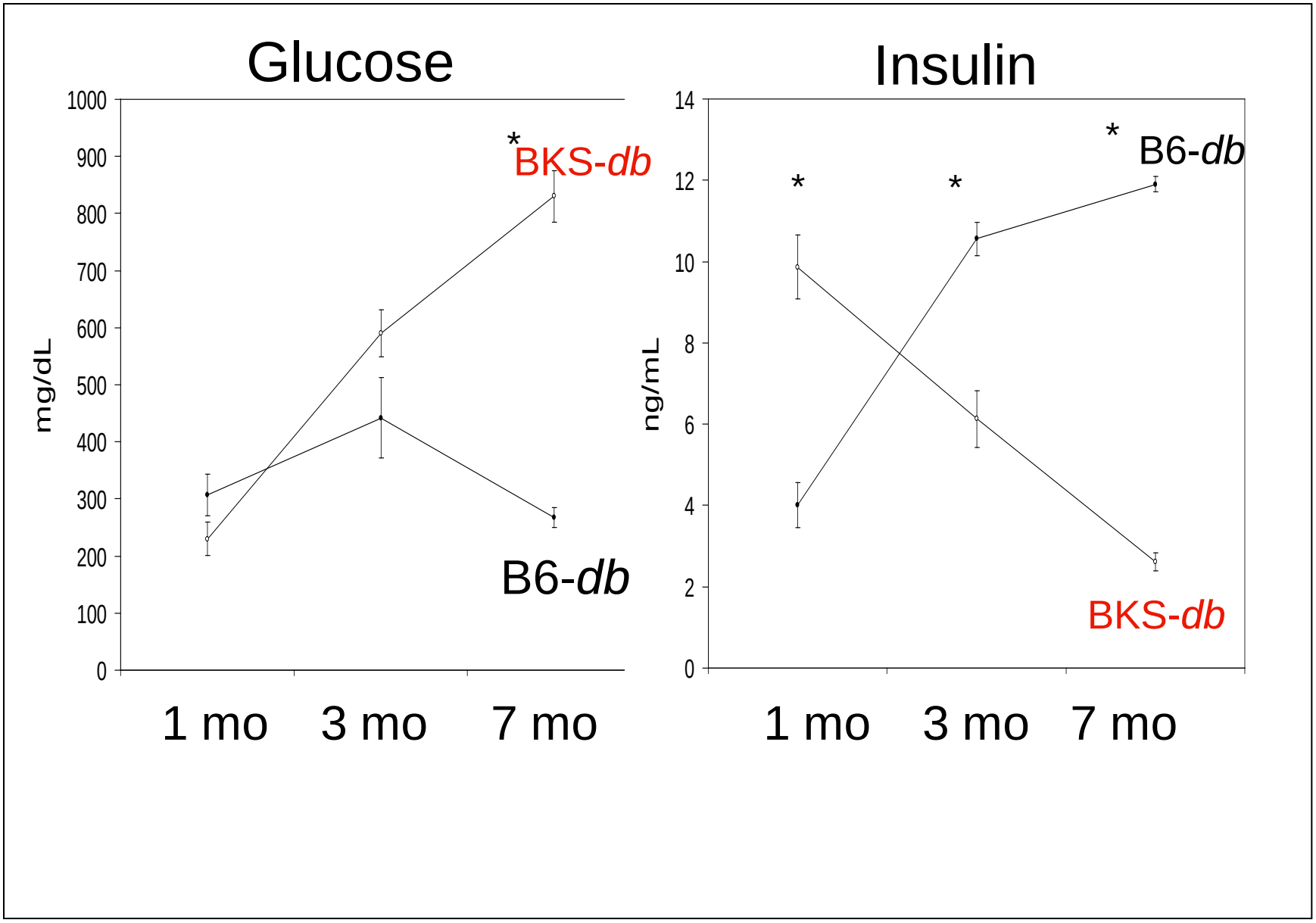
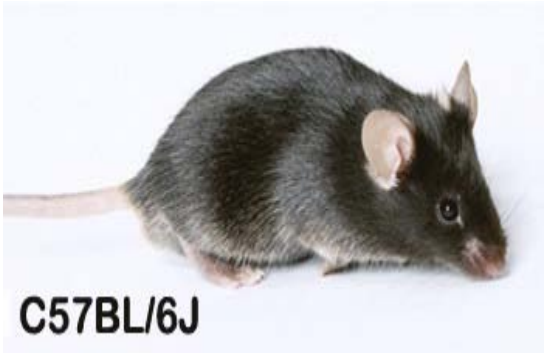


Diabetes Susceptibility: QTL Mapping in

C57BL/6J X DBA/2 db/db F2

B6-*db* vs BKS-*db*: Difference in Diabetes Susceptibility





db/+ male

= Lepr mutation

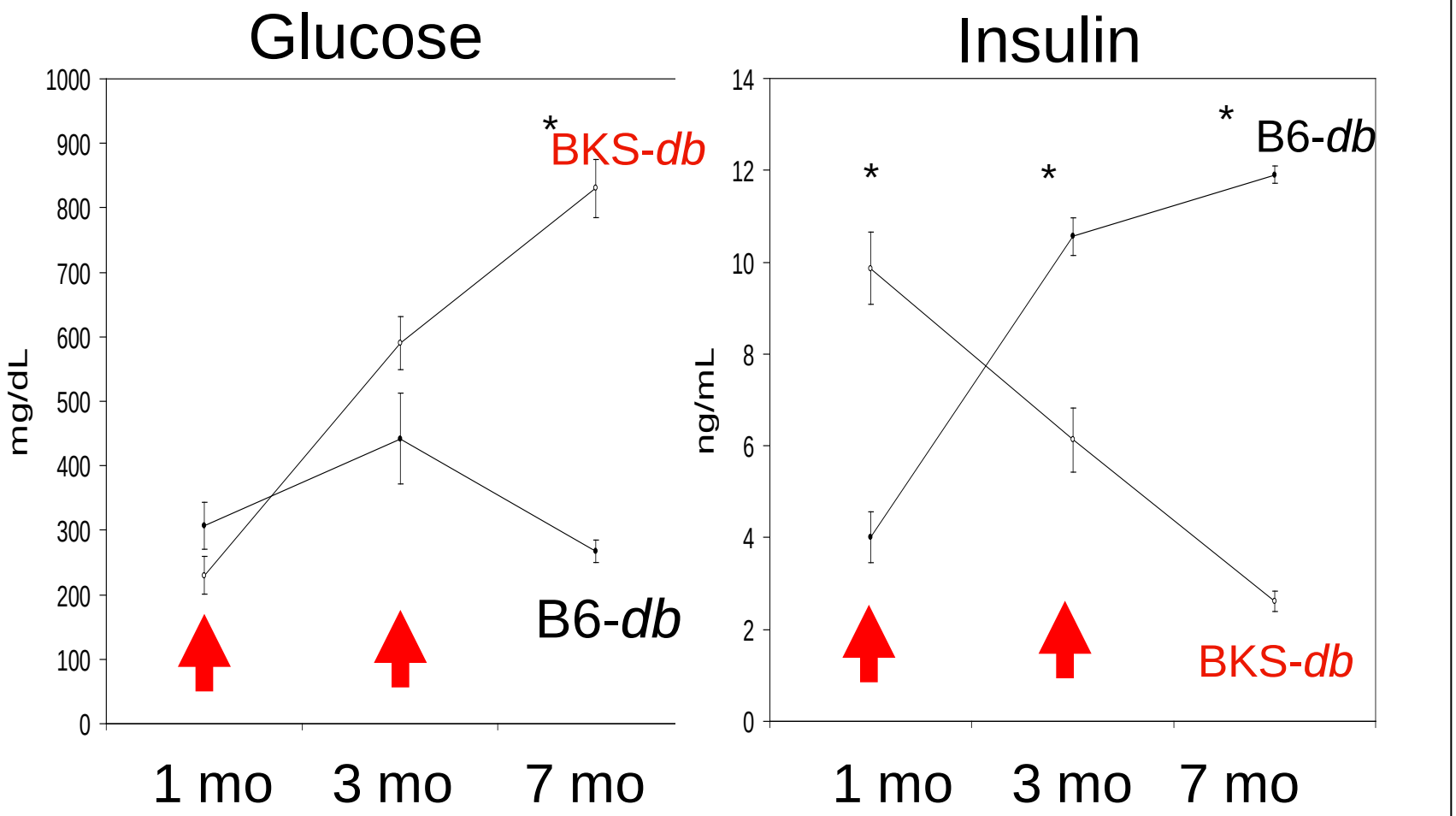
= db

F1

F2

db/db

B6-*db* vs BKS-*db*: Time points for phenotypes



Experiment Design

Goup1:
252 Mice

(normal chow)

Sacrifice 4 weeks of age.
Prediabetic (insulin resistance, beta cell compensation)

week4 → week12

Goup2:
248 Mice

(normal chow)

Plasma Glucose.
Blood proteins measurements:
Insulin, CD40, et.al.

Sacrifice 12 weeks of age.
Diabetic (insulin resistance + incipient beta cell failure)

Phenotypes:

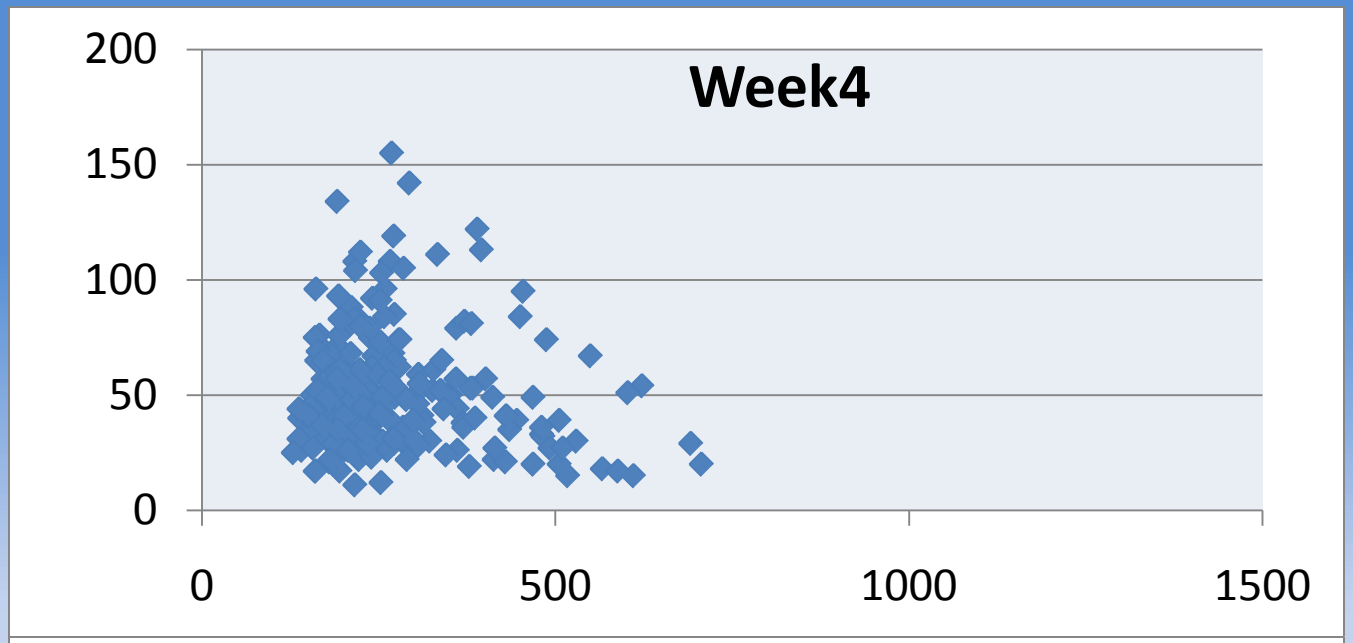
- **General**
  - Plasma glucose & insulin
  - Plasma lipids
  - Body weight
  - Body NMR
    - Lean body mass %
    - Fat body mass %
  - Liver NMR
    - % Lean mass
    - % Fat mass

- **Organ Weights:**
  - Liver
  - Heart
  - Kidneys
  - Spleen
  - Fat depots
    - Gonadal
    - Mesenteric
    - Retroperitoneal
    - Sub Cutaneous
- Many others

Genotypes

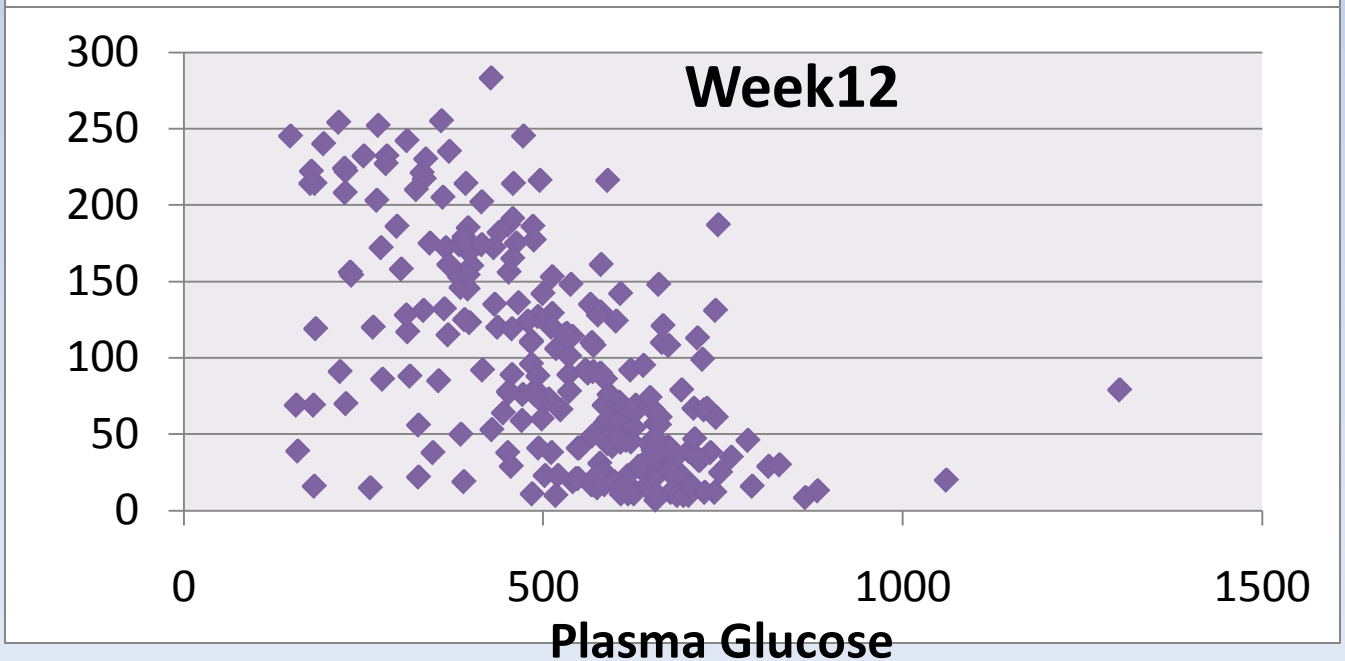
- Affymetrix 5K Mouse SNP panel
  - About 2600 SNPs informative between
    - C57BL/6
    - DBA/2

Plasma Glucose and Insulin



Correlation: -0.11

Adjusted R^2: 0.008



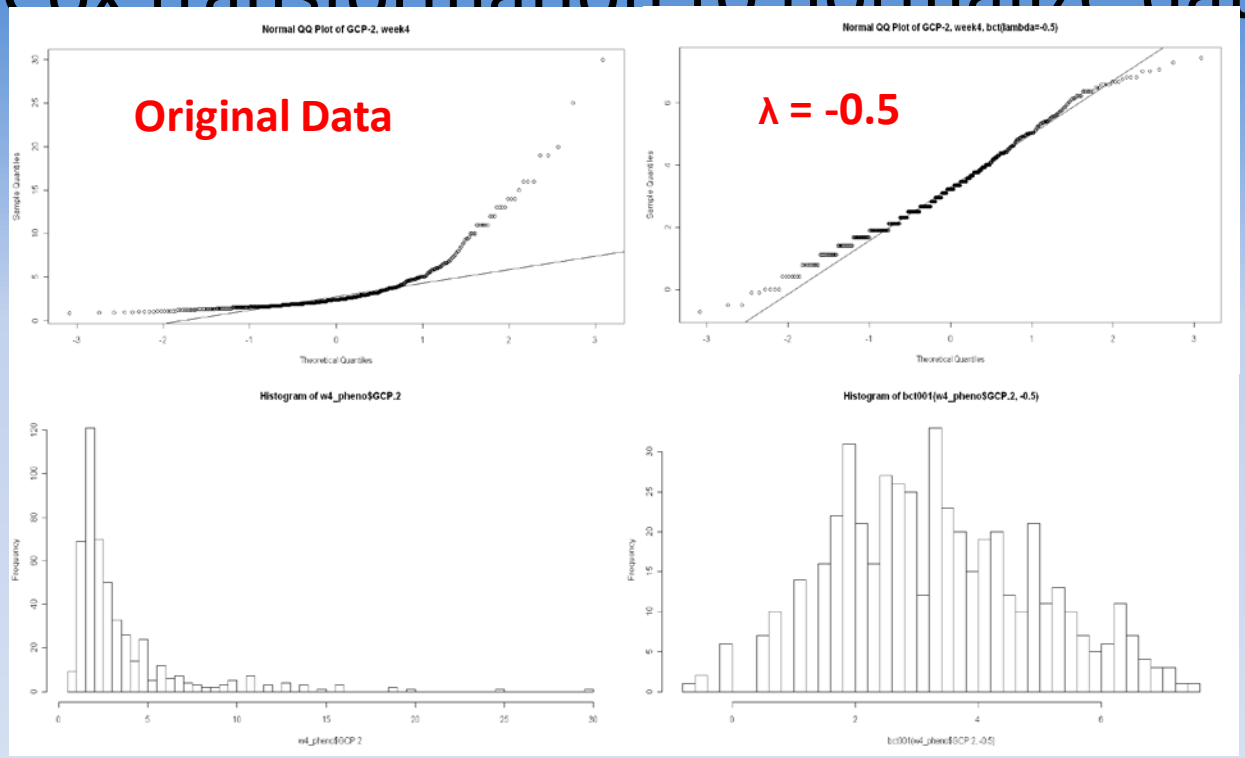
Correlation: -0.59

Adjusted R^2: 0.344

Insulin

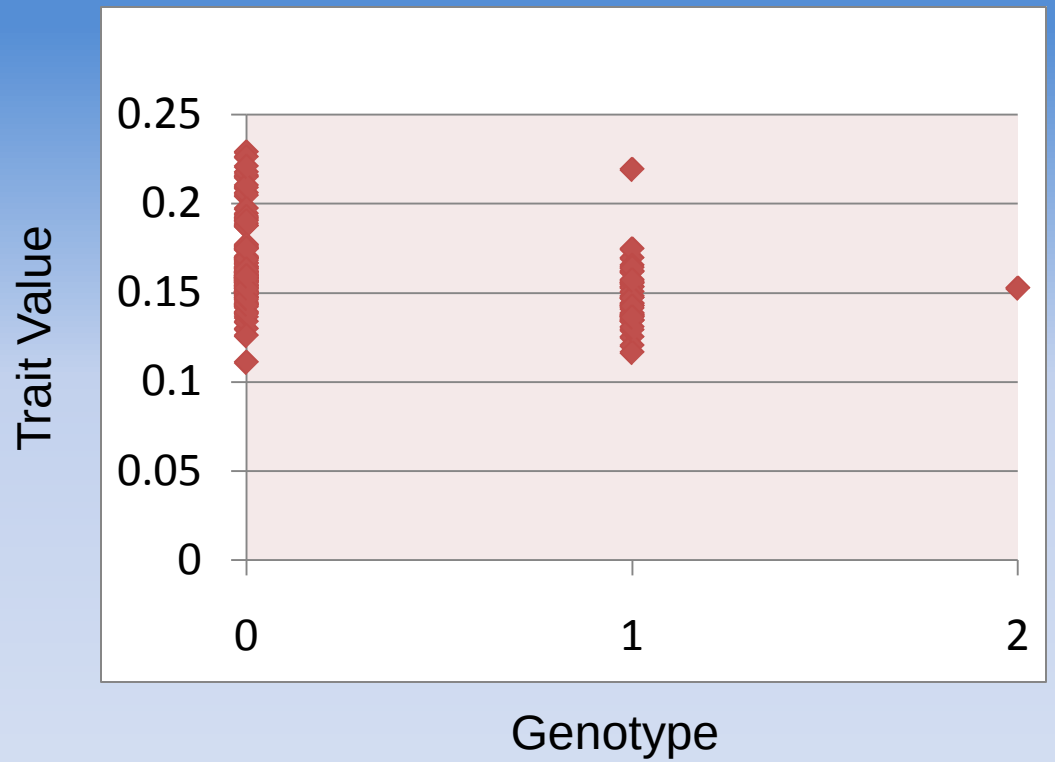
QTL Mapping of Phenotypes

- Box-Cox transformation to normalize data



- Rqtl software for identifying phenotype QTLs

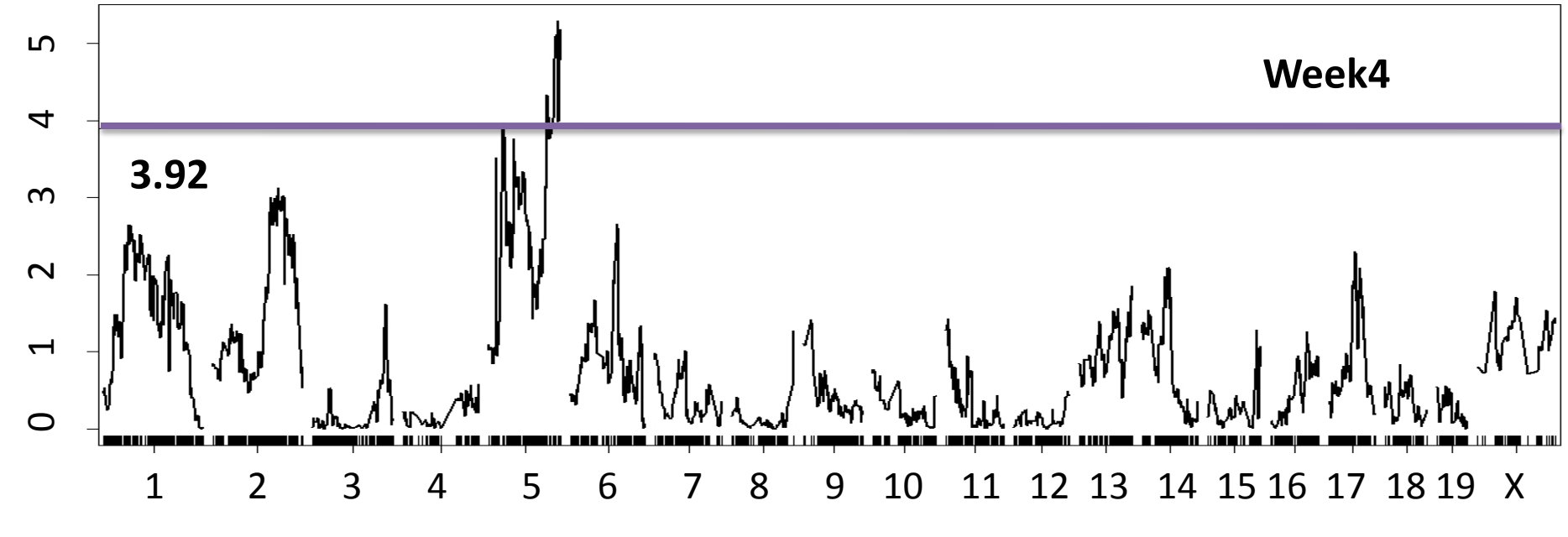
Mapping by Marker Regression



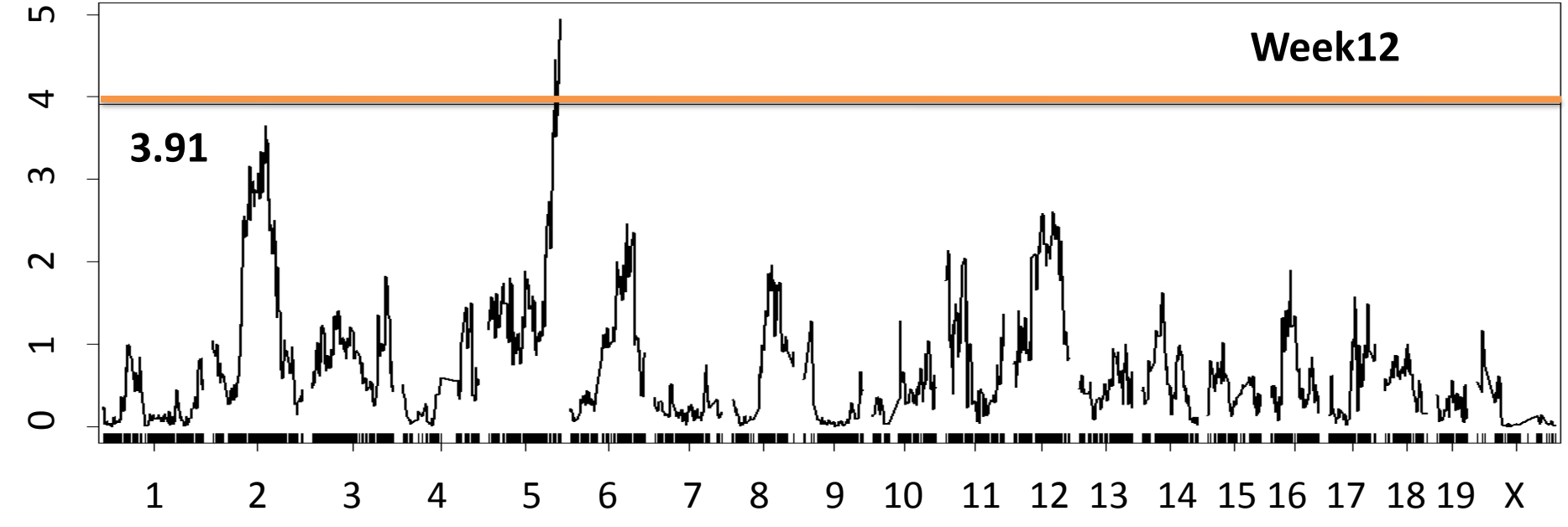
Adjusted R^2: 0.09559
P value: 0.000132 *

0: homozygous allele1, B6-db/B6-db
1: heterozygous alleles, B6-db/DBA
2: homozygous allele2, DBA/DBA

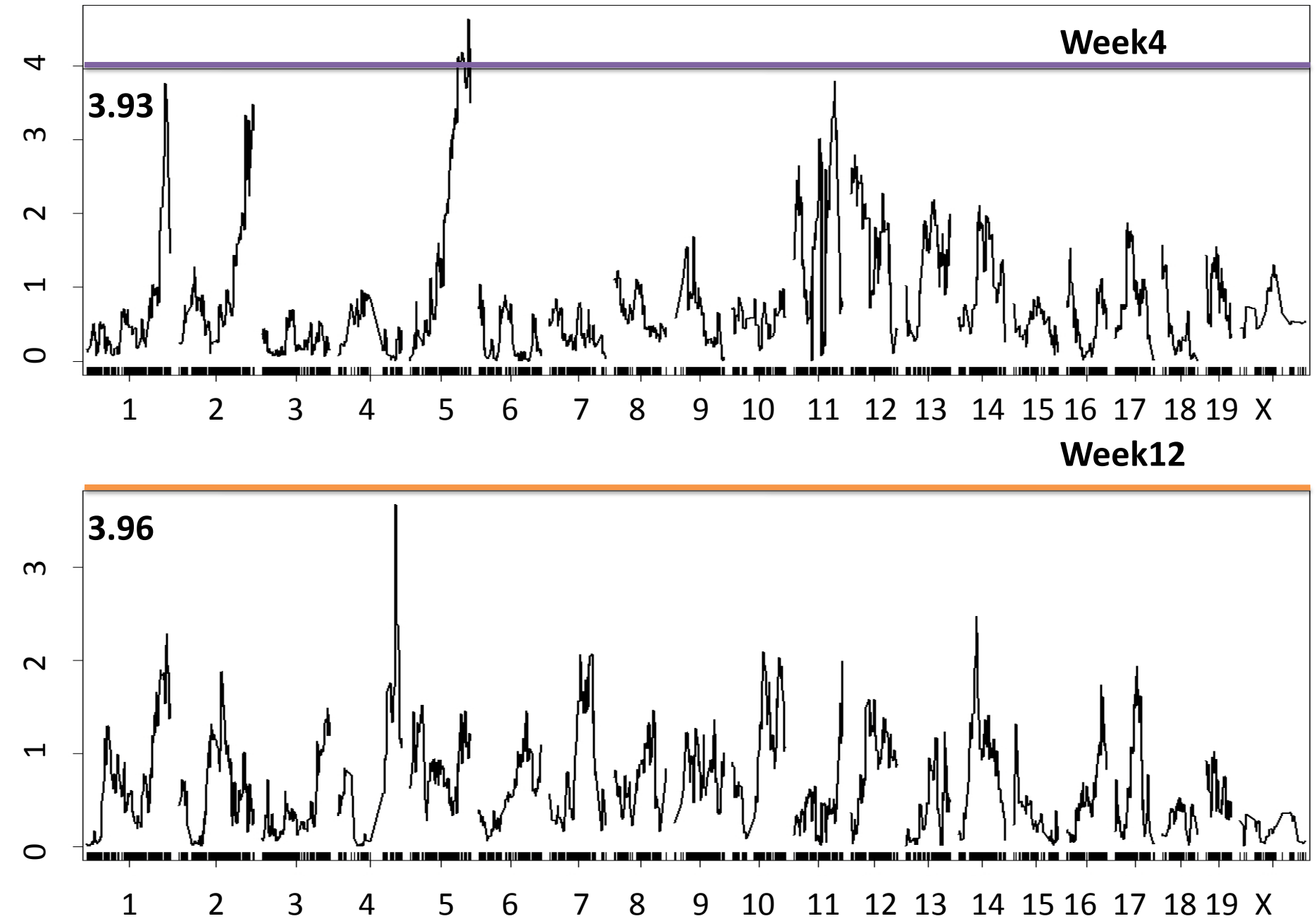
Insulin

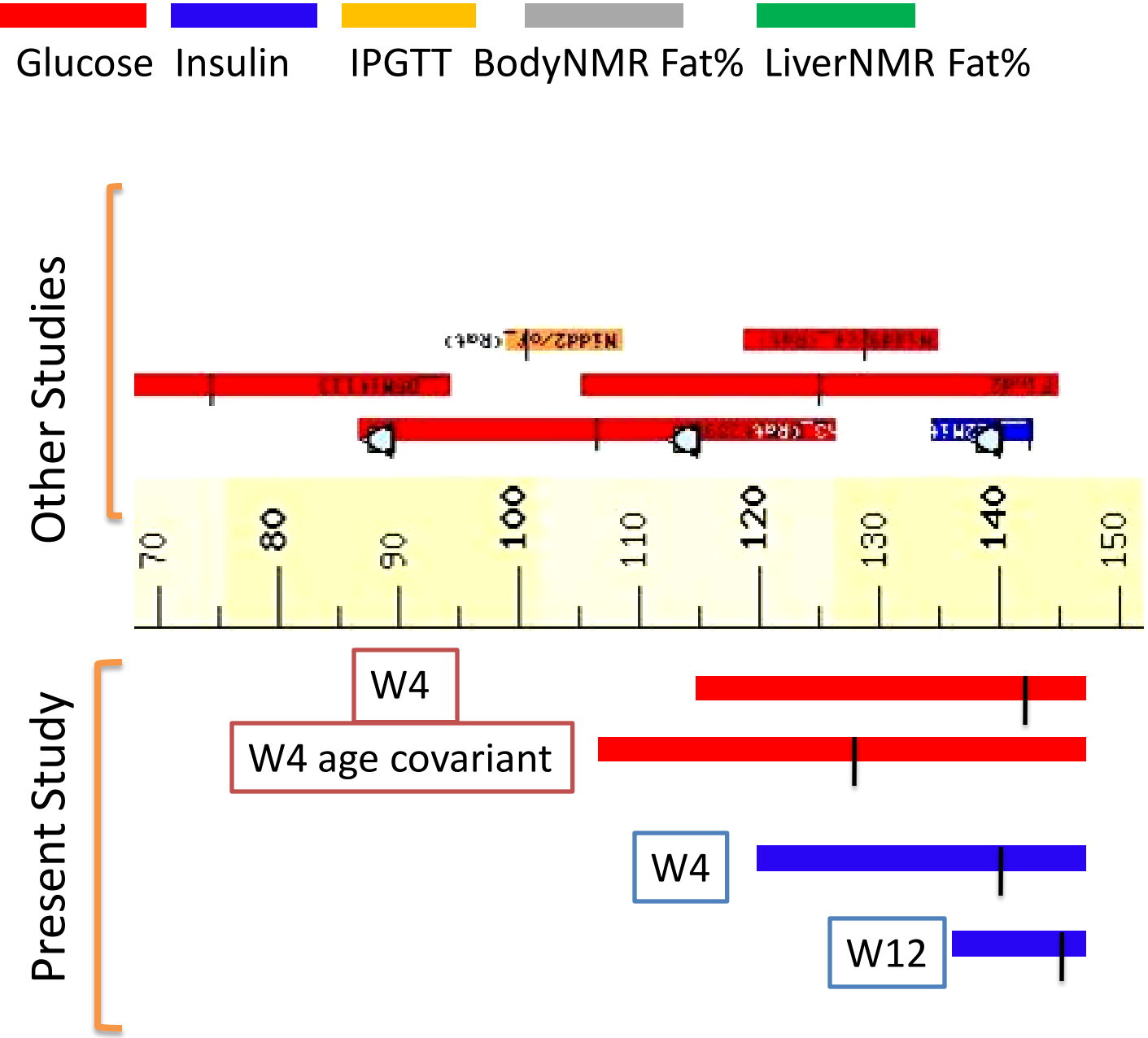


w12_250_Insulin_mr

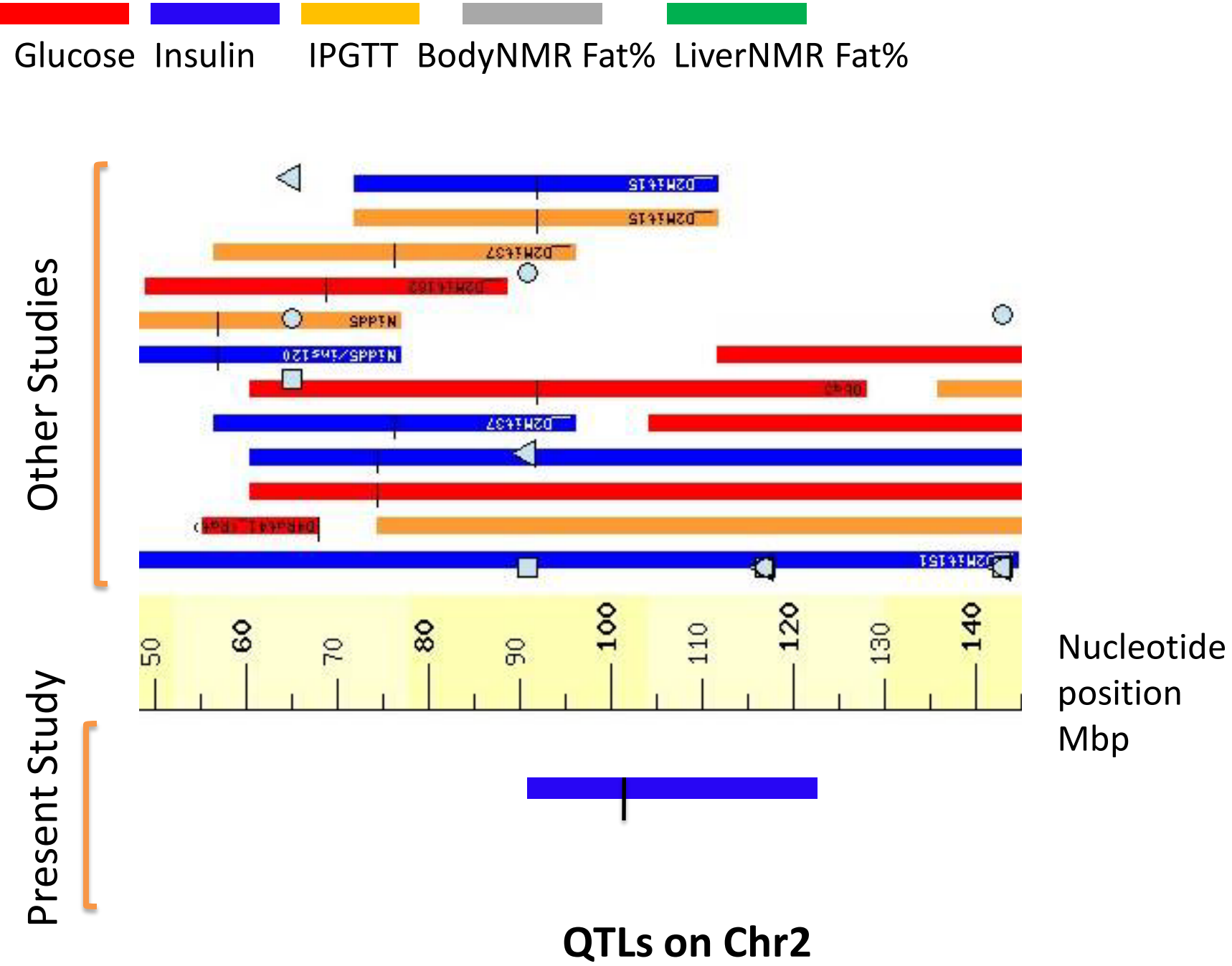


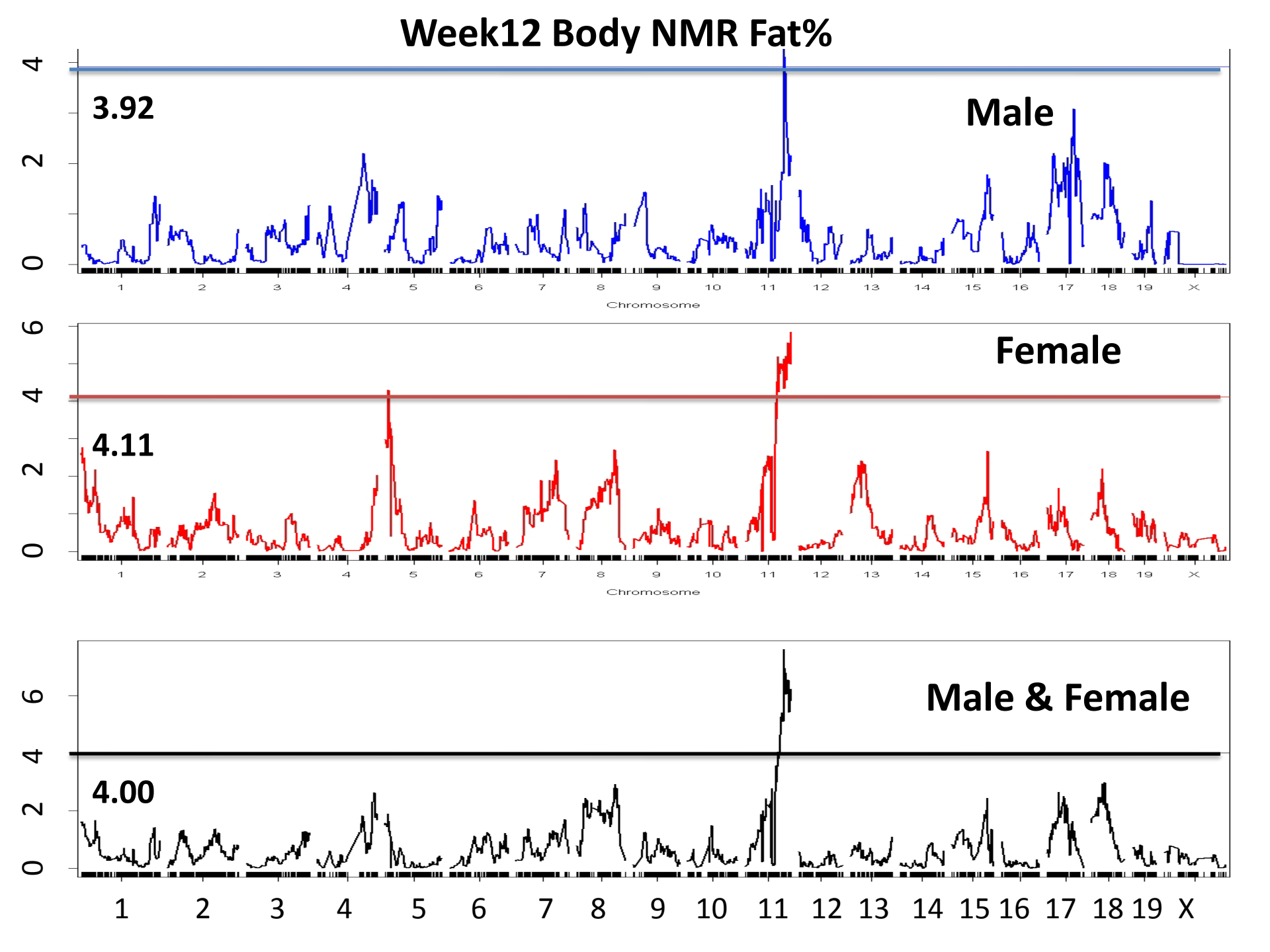
Plasma Glucose mg per dl

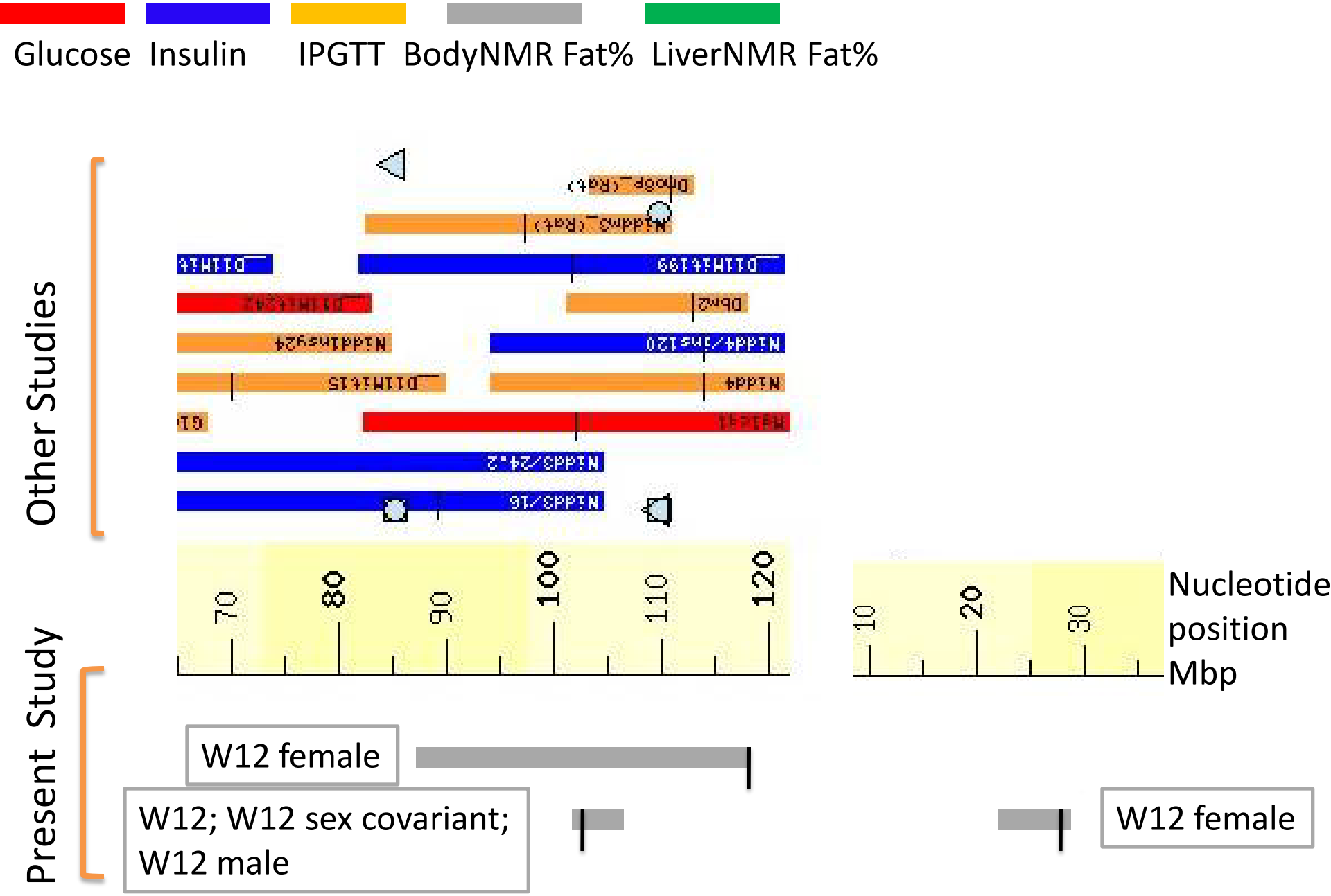




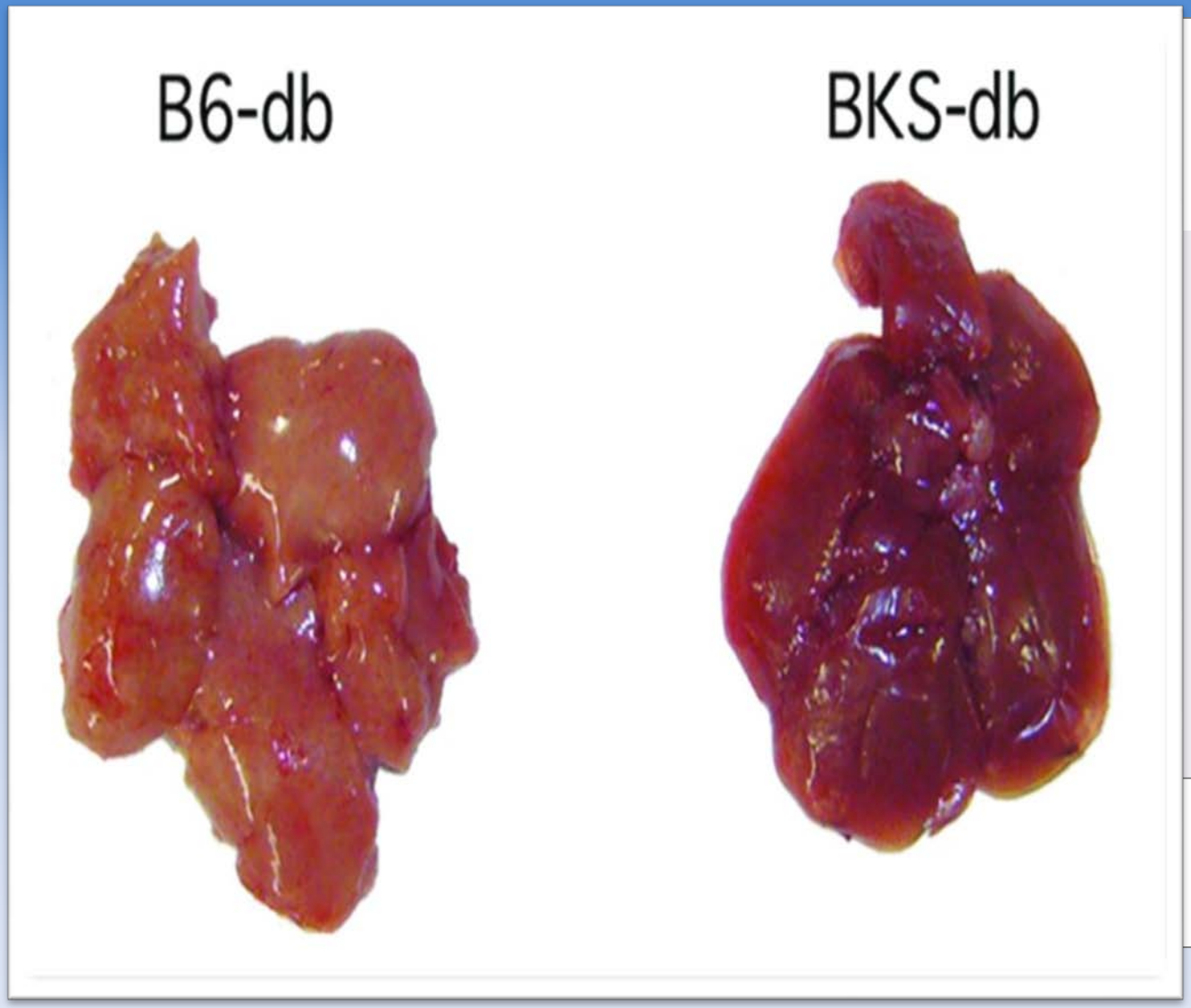
QTLs on distal Chr5

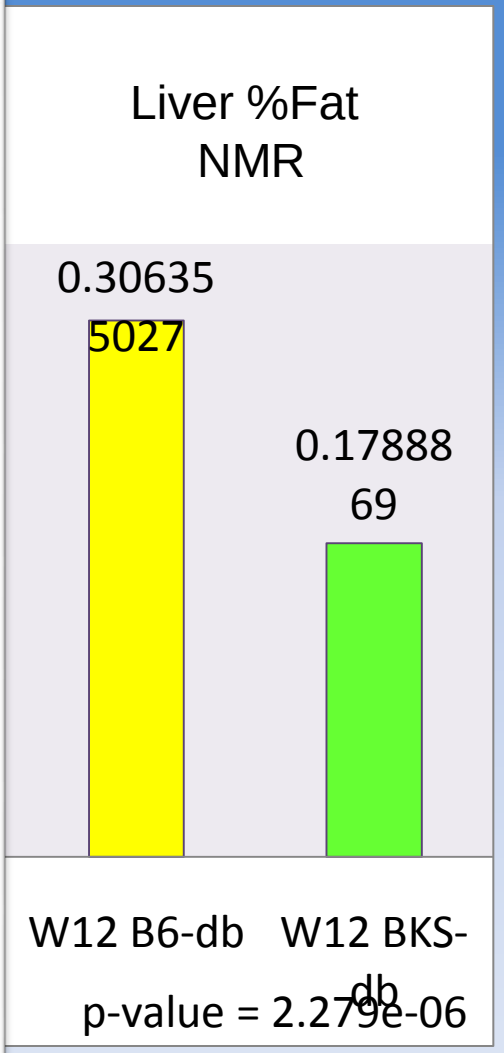

Glucose
Insulin
IPGTT
BodyNMR Fat%
LiverNMR Fat%
Other Studies
Present Study
50
60
70
80
90
100
110
120
130
140
Nucleotide position Mbp
QTLs on Chr2


Week12 Body NMR Fat%
3.92
Male
Chromosome
Female
4.11
Chromosome
Male & Female
4.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X


Glucose
Insulin
IPGTT
BodyNMR Fat%
LiverNMR Fat%
Other Studies
Present Study
70
80
90
100
110
120
10
20
30
Nucleotide position Mbp
W12 female
W12; W12 sex covariant; W12 male
W12 female
QTLs on distal Chr11
QTLs on proximal Chr5

Fat Deposition in the Liver





Hepatic Lipogenesis

- Hepatic lipogenic capacity in diabetes susceptibility
  - Lower fat levels in livers of diabetes-susceptible mice
    - C57BLKS db/db vs C57BL/6 db/db mice
    - BTBR ob/ob vs C57BL/6 ob/ob mice - Alan Attie
      - Suppressed expression of gluconeogenic genes
        - » Diabetes, 2003

P Value Matrix

	LiverNMR Fat%	BodyNMR Fat%	Plasma Glucose mg/dl	Insulin
LiverNMR Fat%	0	0.0415481	0.0087262	0.0276204
BodyNMR Fat%	0.0888225	0	6.18E-10	0.0927811
Plasma Glucose mg/dl	2.60E-05	0.2665759	0	0.0919955
Insulin	7.74E-06	0.0346712	2.36E-24	0

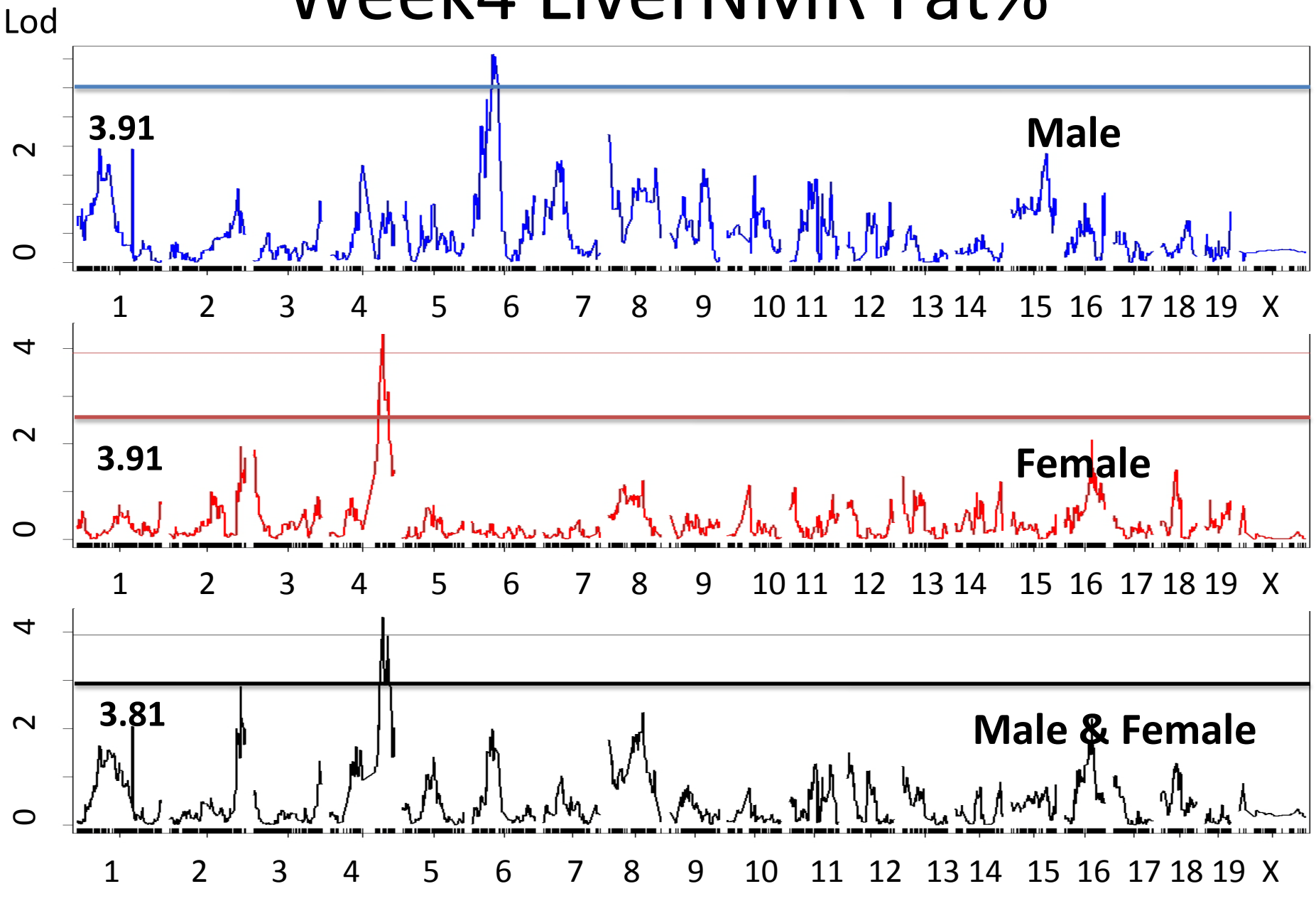
W12 *W4*

Correlation Coefficient Matrix

	LiverNMR Fat%	BodyNMR Fat%	Plasma Glucose mg/dl	Insulin
LiverNMR Fat%	1	0.1300494	0.1668865	0.1404534
BodyNMR Fat%	0.1087247	1	0.3813249	0.1074075
Plasma Glucose mg/dl	-0.265212	0.0711061	1	-0.107665
Insulin	0.2808103	-0.134466	-0.588863	1

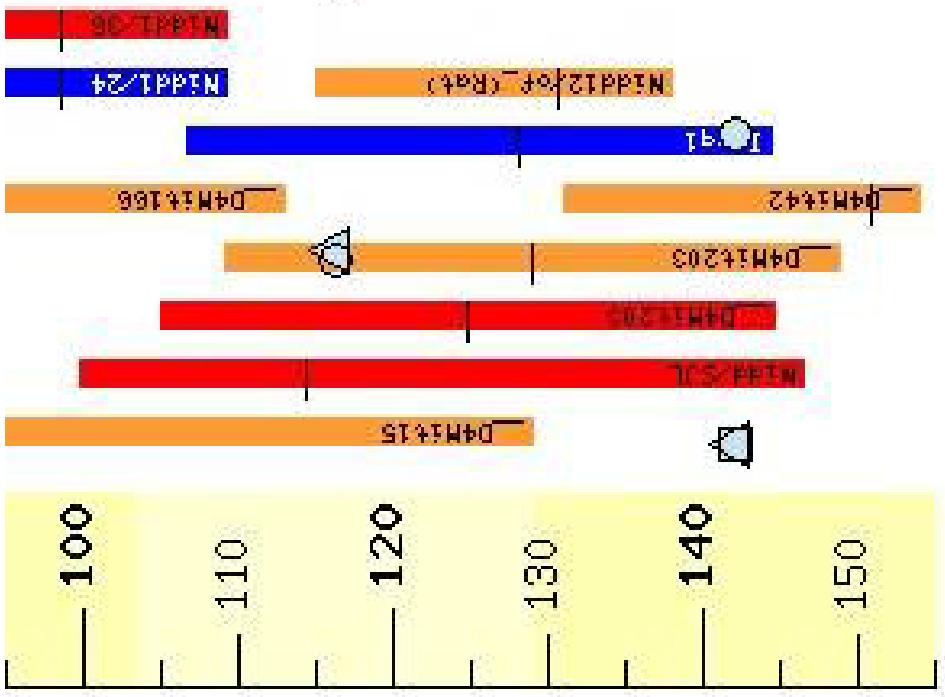
W12 *W4*

Week4 LiverNMR Fat%



Glucose Insulin IPGTT BodyNMR Fat% LiverNMR Fat%

Other Studies



Nucleotide position, Mbp

Present Study

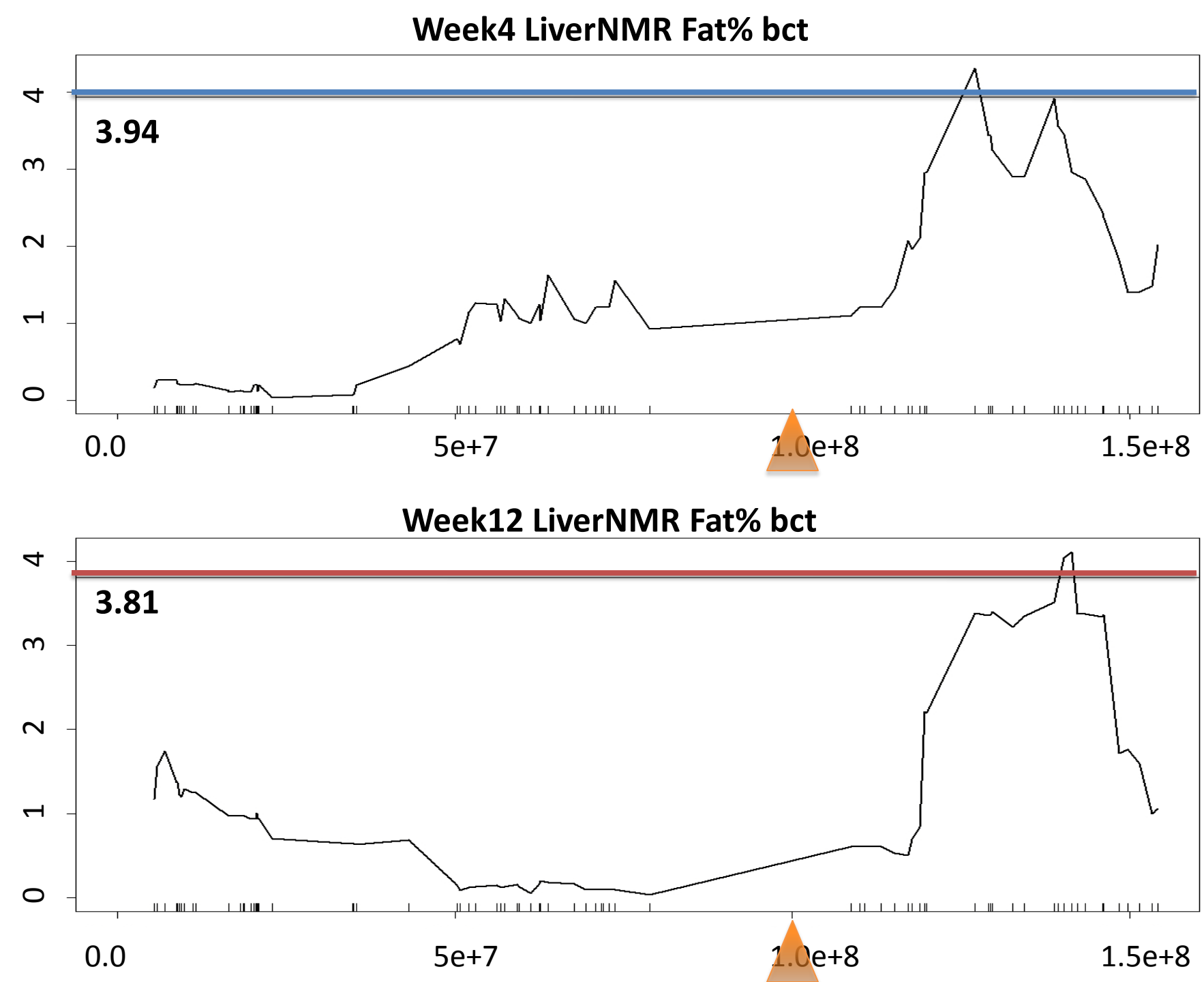
W4 bct; W4 bct sex covariant

W12 bct; W12 bct sex covariant

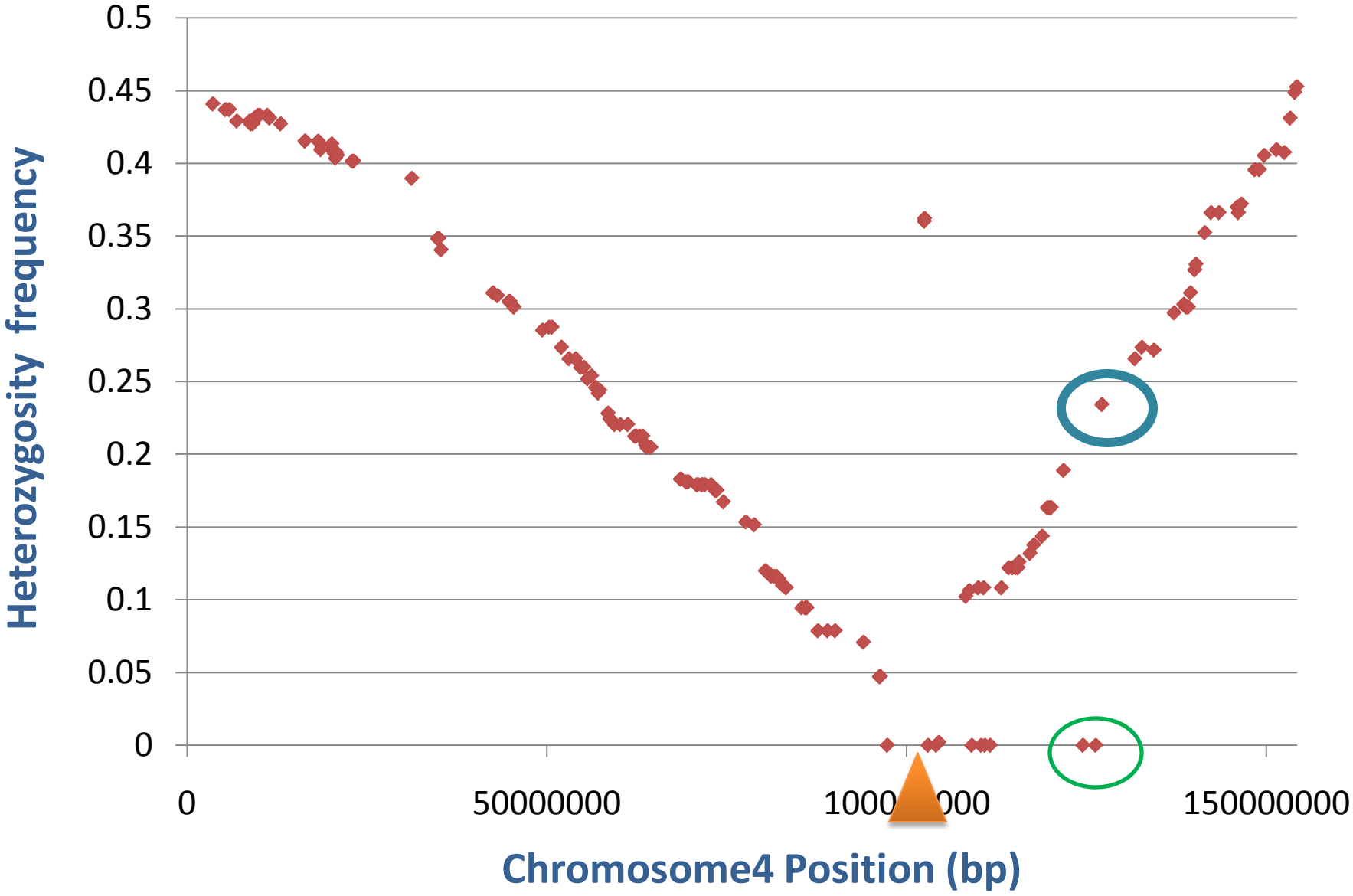
QTLs on distal Chr4

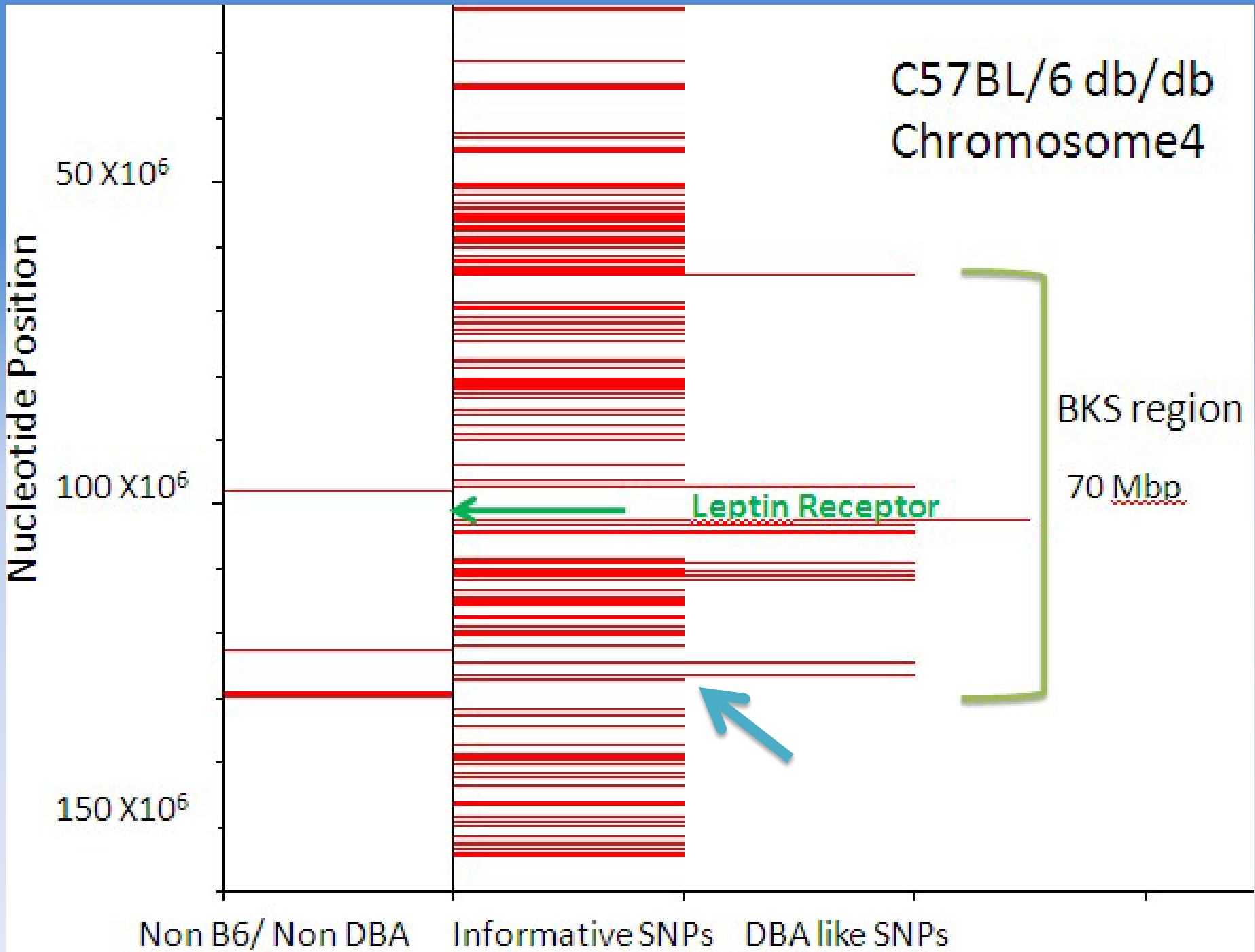
Going Forward

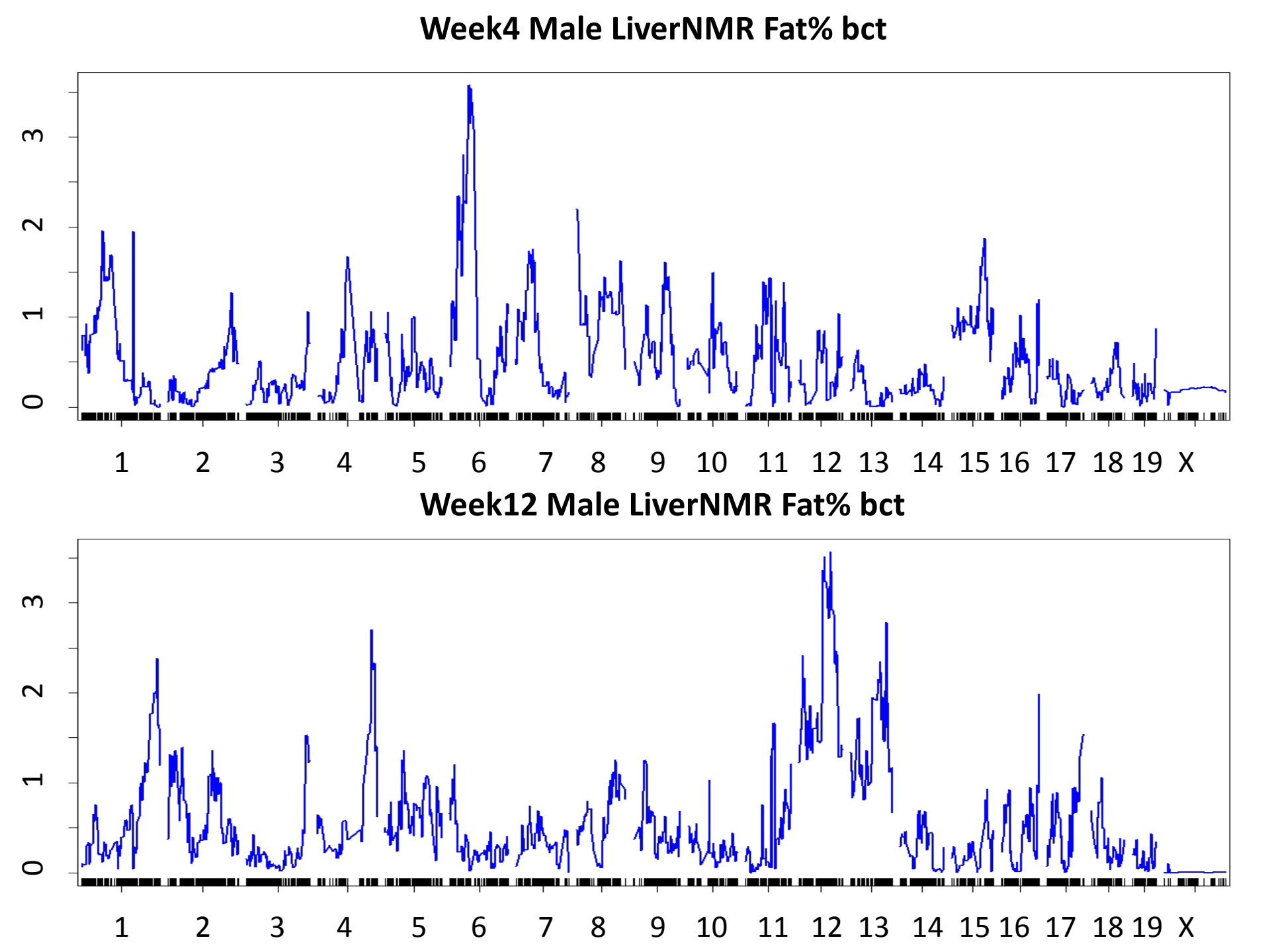
- Incorporate expression data to
  - Identify gene clusters associated with phenotypes
  - Analyze for underlying pathways
    - Enrichment for pathway-specific genes
    - Genes in clusters with cis-acting eQTLs that match phenotypic QTL's
  - Analyze for tissue-tissue interactions that impact the diabetic phenotype
    - Adipose – Liver
    - Adipose – Islets
  - Analyze for phenotype and expression QTLs that arise in the DBA regions of the BKS genome.


Week4 LiverNMR Fat% bct
3.94
0
1
2
3
4
0.0
5e+7
1.0e+8
1.5e+8
Week12 LiverNMR Fat% bct
3.81
0
1
2
3
4
0.0
5e+7
1.0e+8
1.5e+8

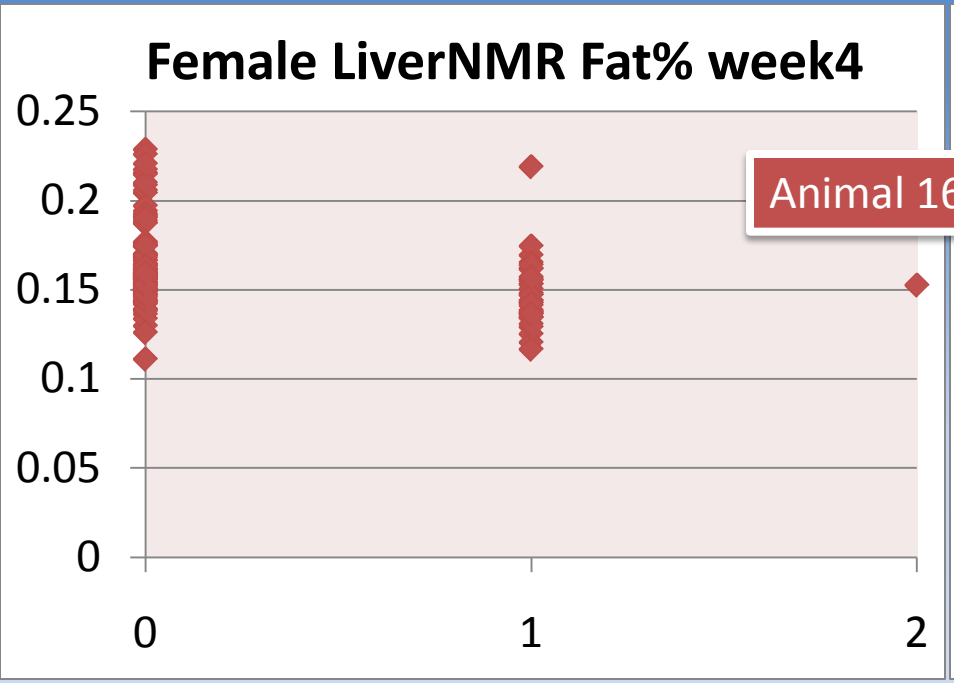
Chr4 Heterozygosity Plot

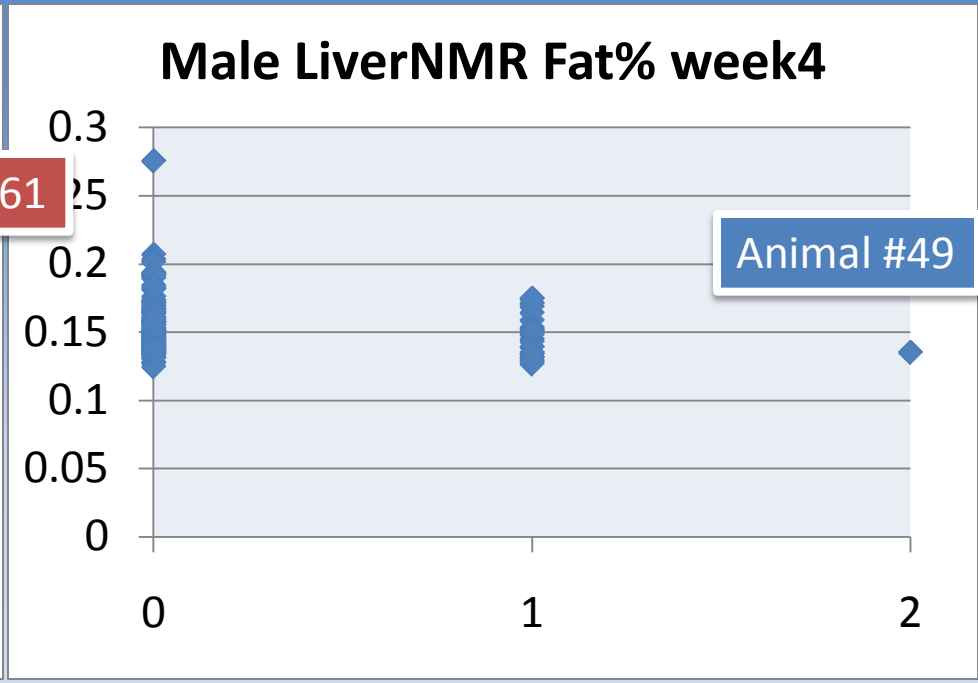



C57BL/6 db/db
Chromosome4
Nucleotide Position
50 X10⁶
100 X10⁶
150 X10⁶
BKS region
70 Mbp
Leptin Receptor
Non B6/ Non DBA
Informative SNPs
DBA like SNPs


Week4 Male LiverNMR Fat% bct
0
1
2
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
Week12 Male LiverNMR Fat% bct
0
1
2
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

LiverNMR Fat% regression on marker rs13477972





Adjusted R-squared: 0.09559
P value: 0.000132 *

Adjusted R-squared: 0.02278
P value: 0.0635

0: homozygous allele1, B6-db/B6-db
1: heterozygous alleles, B6-db/DBA
2: homozygous allele2, DBA/DBA

Results & Discussion

- Correlation between phenotypes.
- Chr4 db locus.
- QTL mapping. Four traits:
  - Normalize phenotype data: box-cox transform.
  - Mapping four traits: LiverNMR Fat%, BodyNMR Fat%, Plasma Glucose, Insulin.
- Compare QTLs to other studies.

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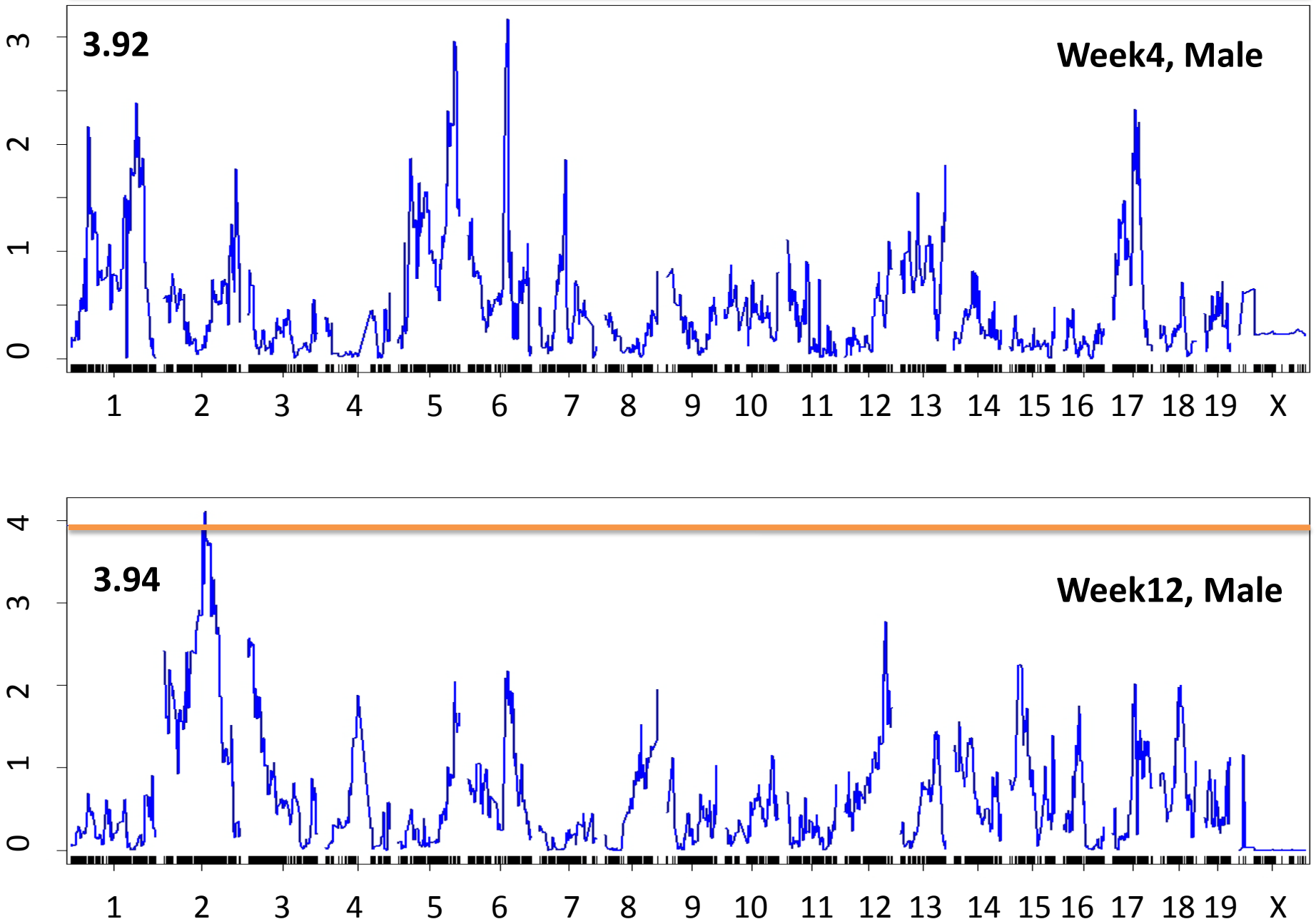
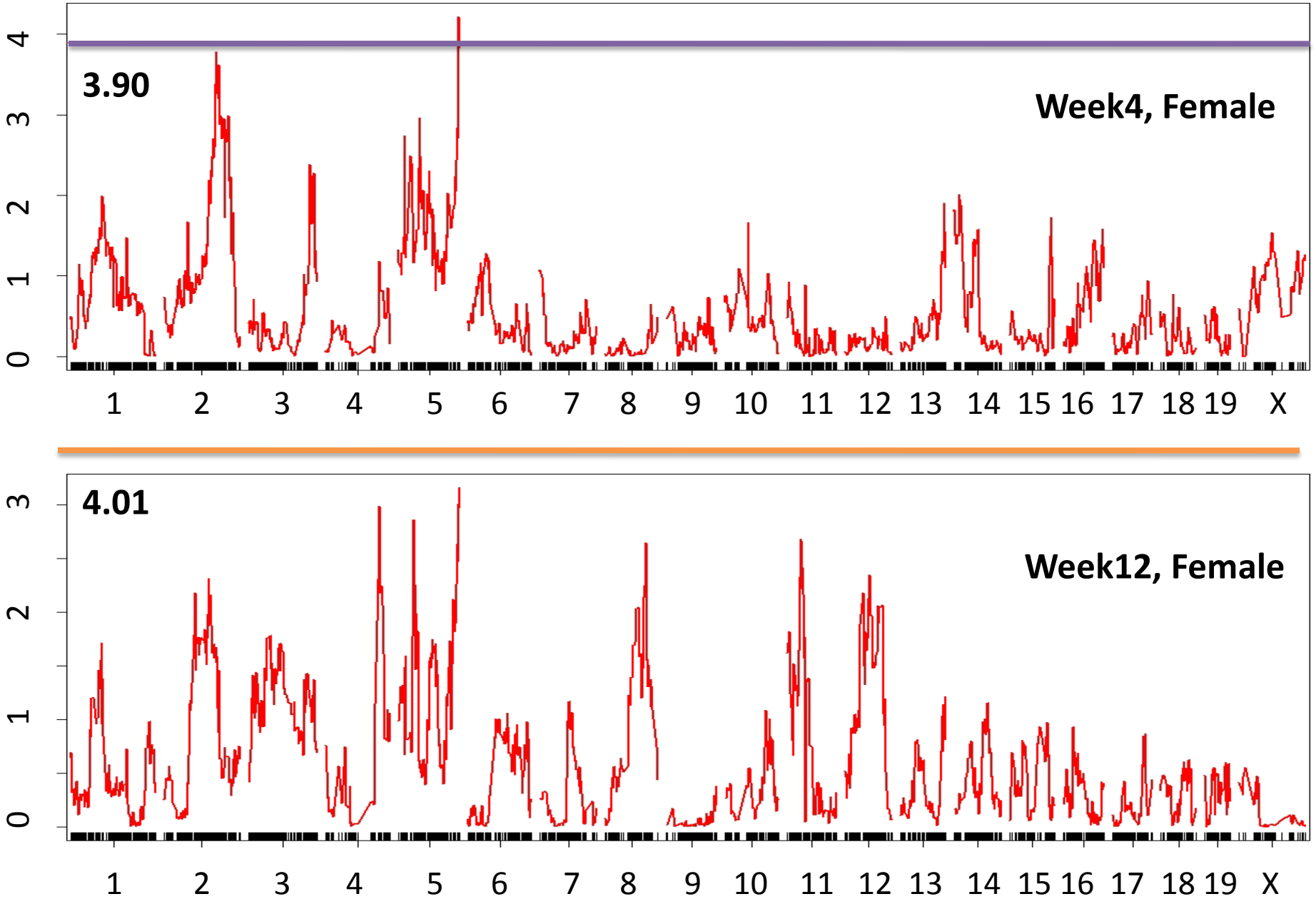
Insulin


3.92
Week4, Male
0
1
2
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
3.94
Week12, Male
0
1
2
3
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

Insulin


3.90
Week4, Female
0
1
2
3
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
4.01
Week12, Female
0
1
2
3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X

Box-Cox Transformation

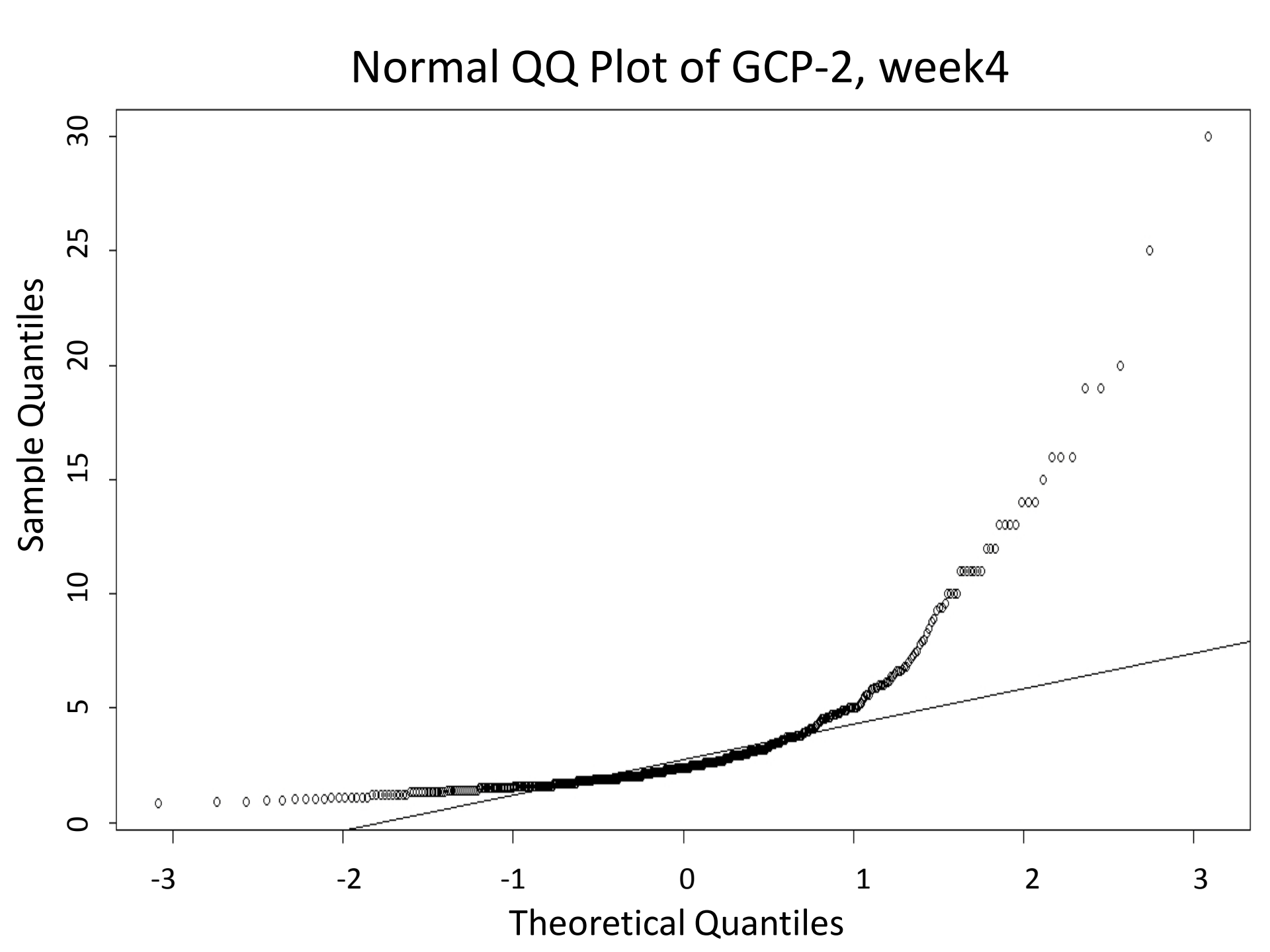
What is the Box-Cox transformations (bct)?

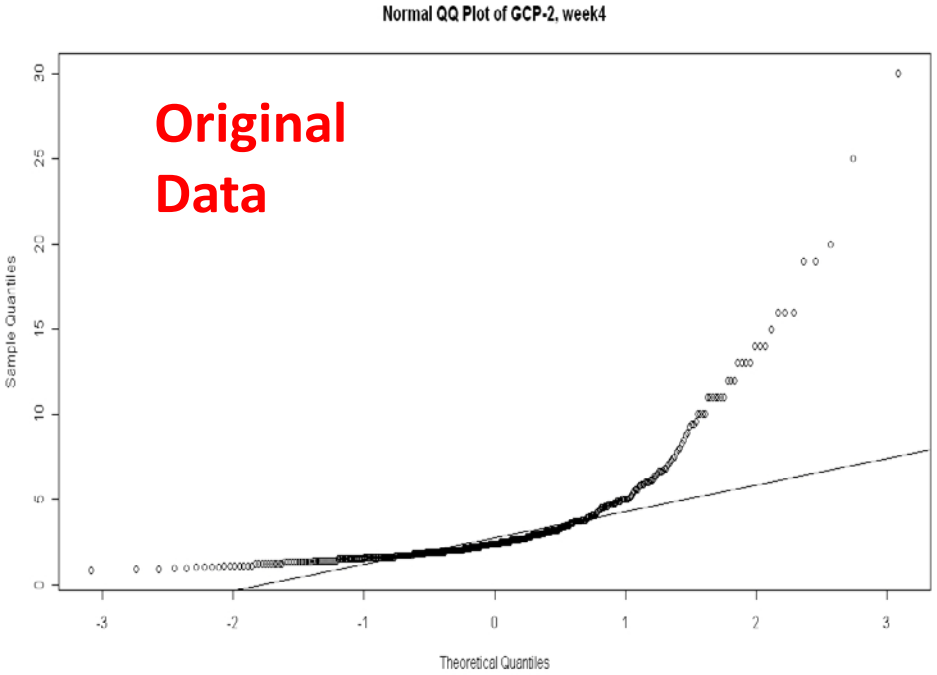
$$y(\lambda) = \begin{cases} \frac{y^\lambda - 1}{\lambda}, & \text{if } \lambda \neq 0; \\ \log y, & \text{if } \lambda = 0. \end{cases} \qquad y(\boldsymbol{\lambda}) = \begin{cases} \frac{(y+\lambda_2)^{\lambda_1} - 1}{\lambda_1}, & \text{if } \lambda_1 \neq 0; \\ \log(y + \lambda_2), & \text{if } \lambda_1 = 0. \end{cases}$$

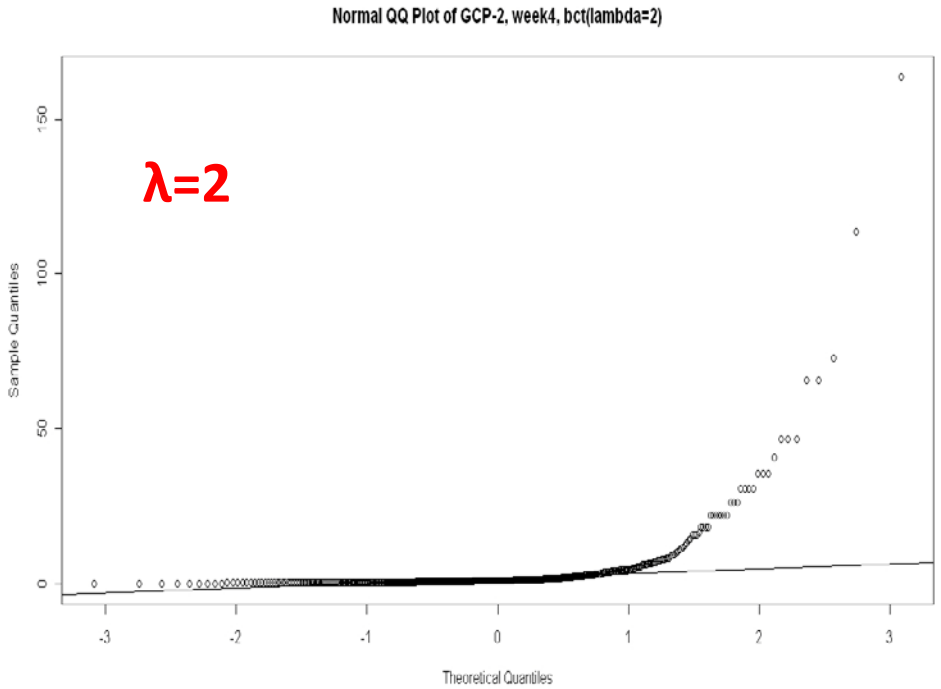
In R, bct(TeachingDemos), there is a little difference:

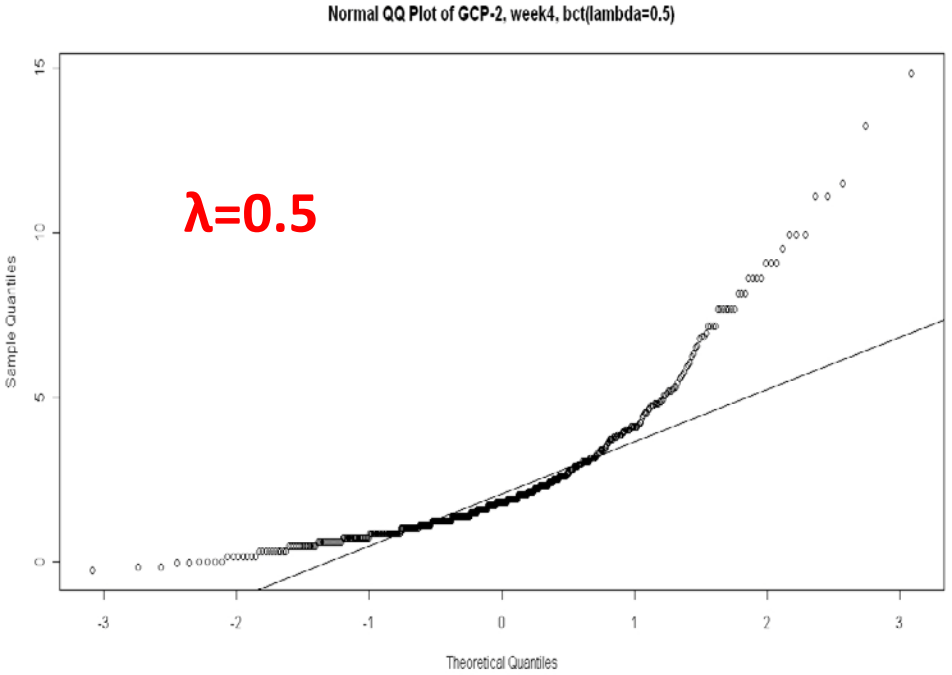
$$\mathrm{y}(\lambda) \begin{cases} \dfrac{y^\lambda - 1}{\lambda \times gm^{\lambda-1}}, \lambda \neq 0 \\ \log(\mathrm{y}) \times \mathrm{gm}, \lambda = 0 \end{cases}$$

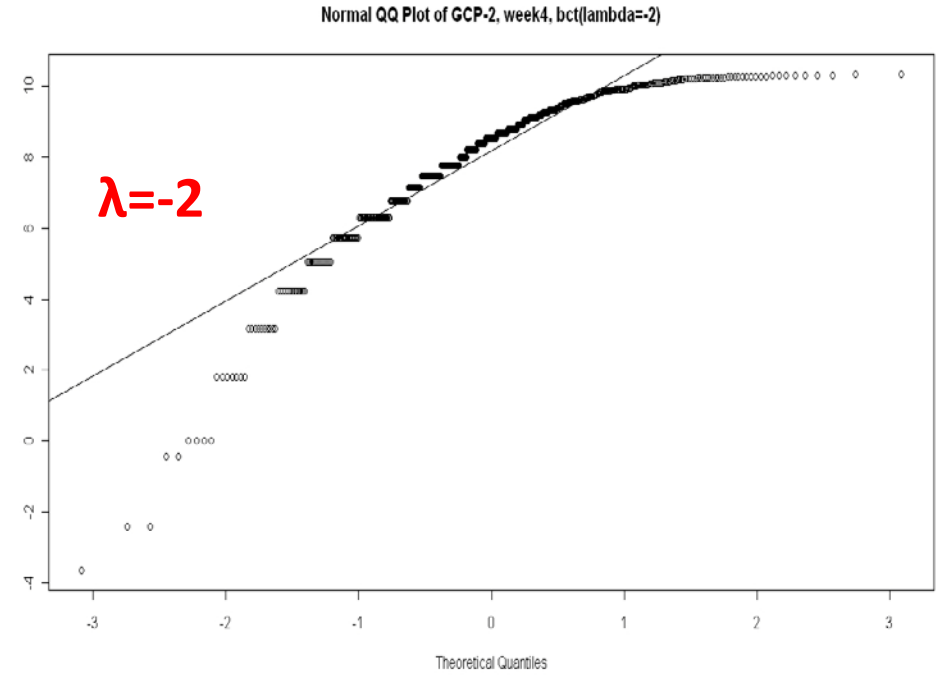
where gm is the geometric mean of the y's

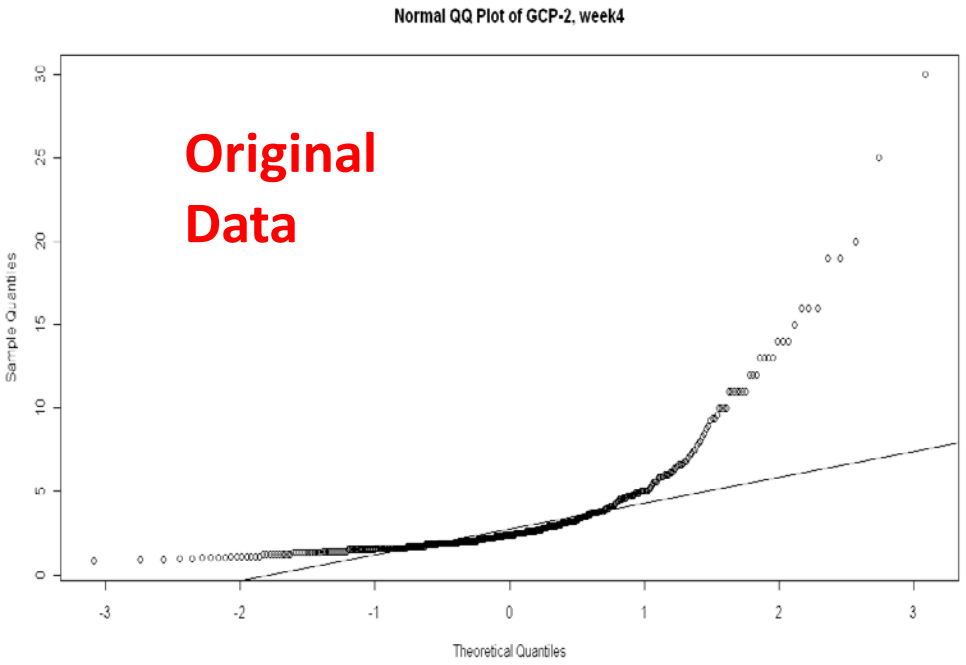

Normal QQ Plot of GCP-2, week4
Sample Quantiles
0
5
10
15
20
25
30
-3
-2
-1
0
1
2
3
Theoretical Quantiles

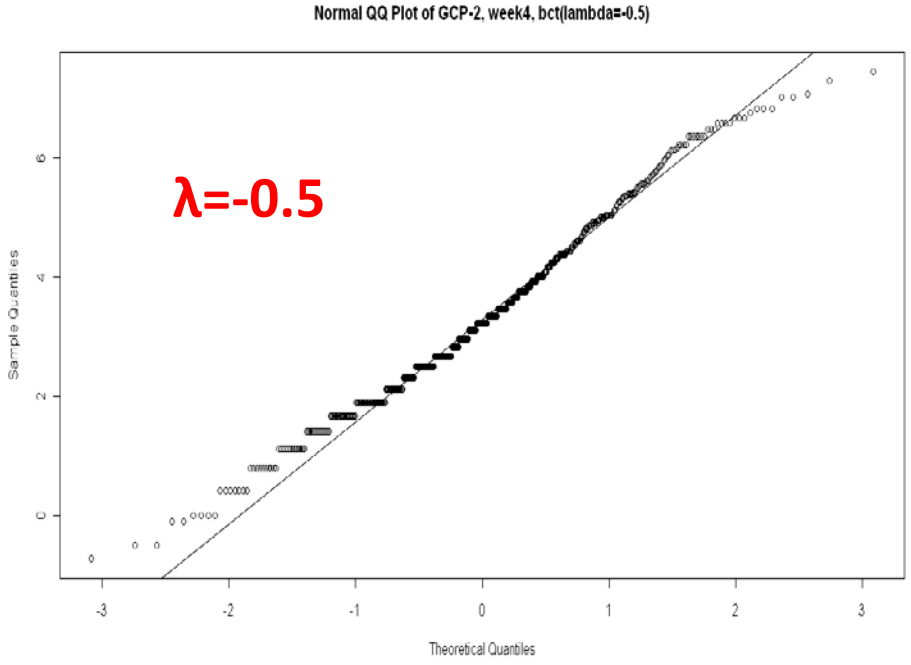

Normal QQ Plot of GCP-2, week4
Original Data
Sample Quantiles
Theoretical Quantiles

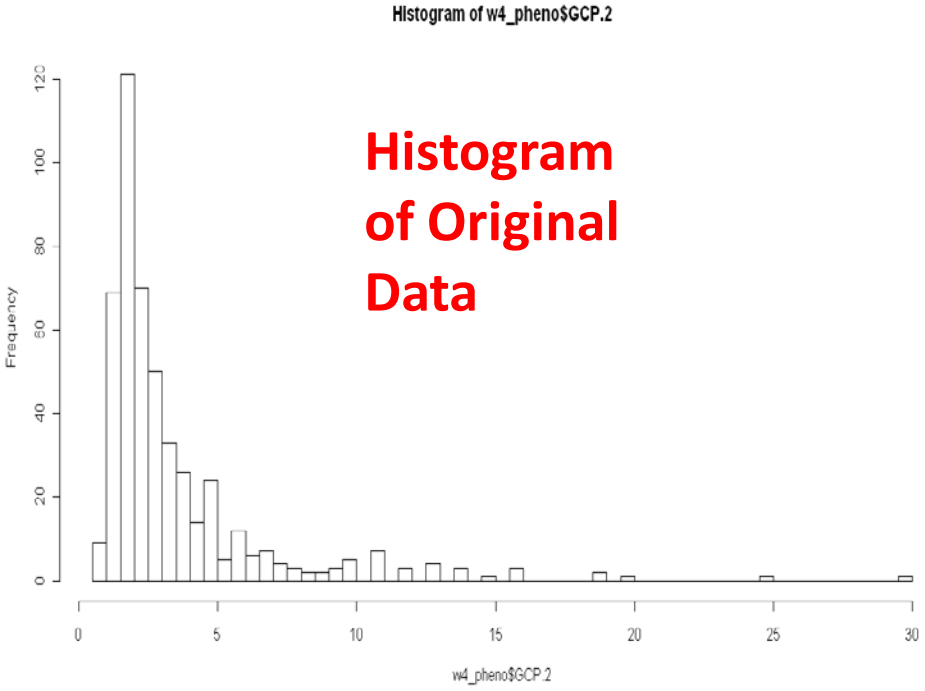

Normal QQ Plot of GCP-2, week4, bct(lambda=2)
λ=2
Sample Quantiles
Theoretical Quantiles

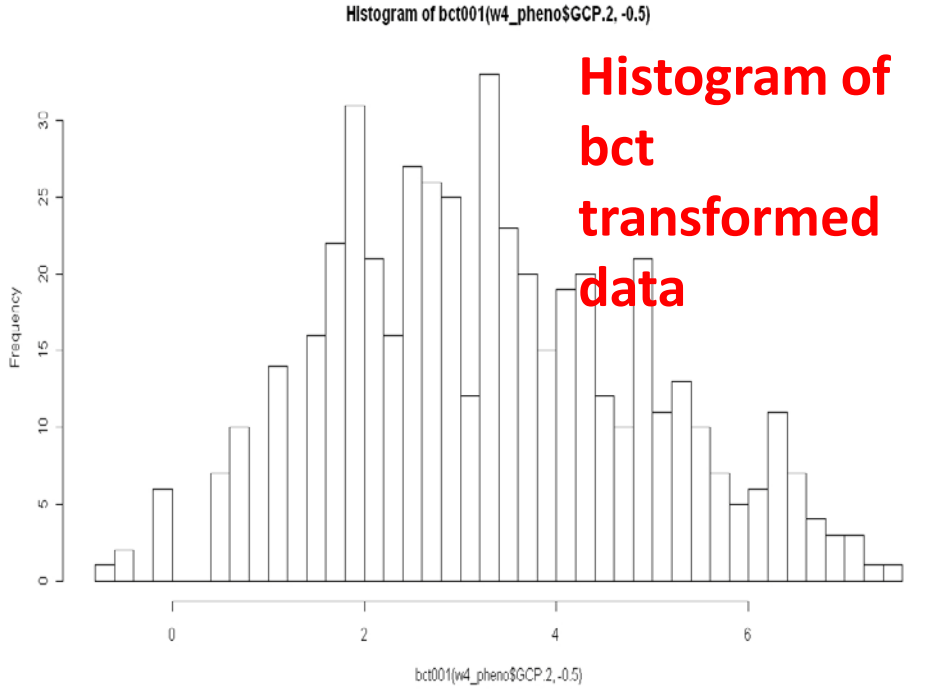

Normal QQ Plot of GCP-2, week4, bct(lambda=0.5)
λ=0.5
Sample Quantiles
Theoretical Quantiles

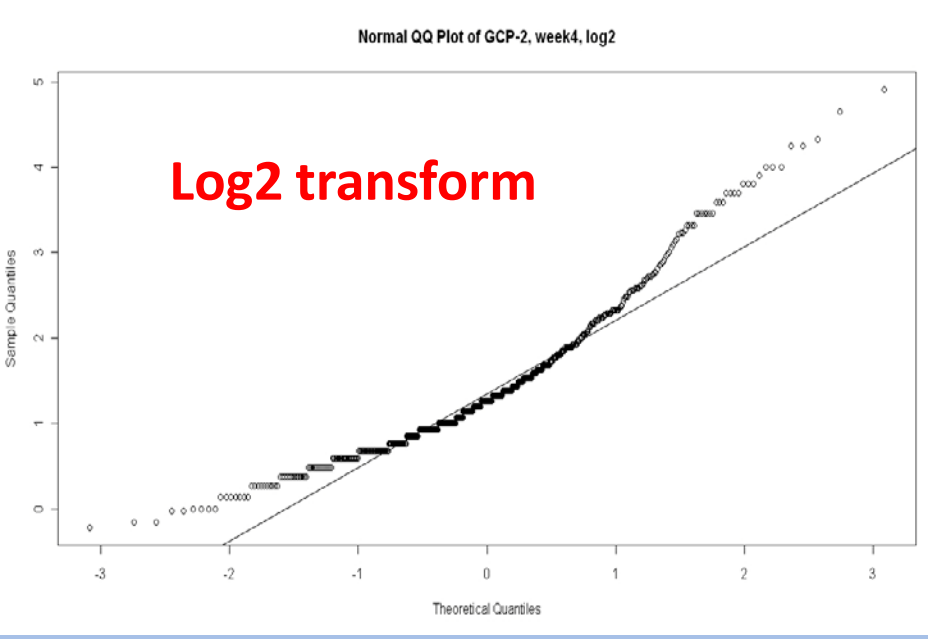

Normal QQ Plot of GCP-2, week4, bct(lambda=-2)
λ=-2
Theoretical Quantiles

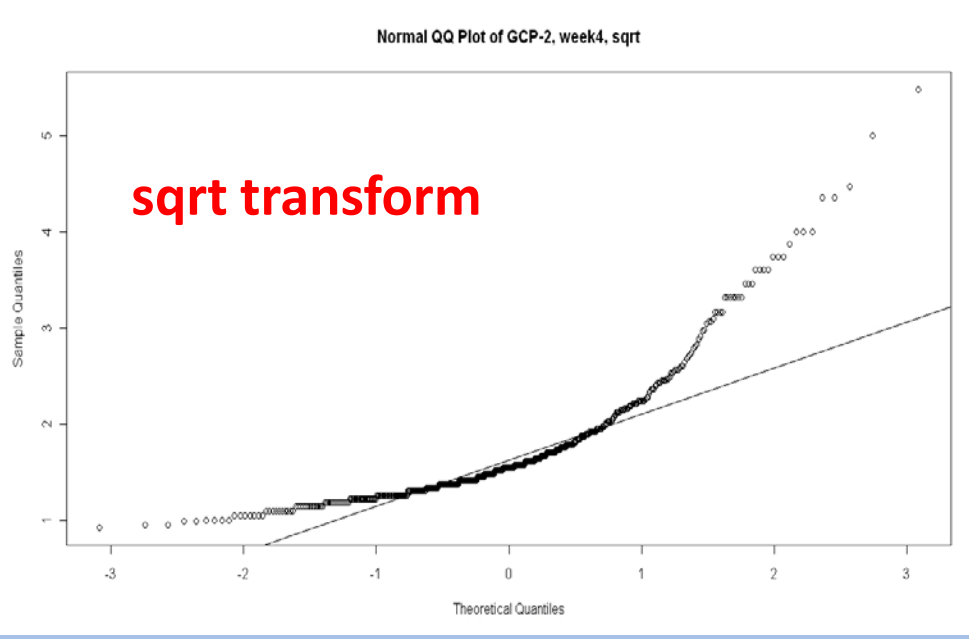

Normal QQ Plot of GCP-2, week4
Original Data
Sample Quantiles
Theoretical Quantiles


Normal QQ Plot of GCP-2, week4, bct(lambda=-0.5)
λ=-0.5
Sample Quantiles
Theoretical Quantiles


Histogram of w4_pheno$GCP.2
Histogram of Original Data
Frequency
w4_pheno$GCP.2

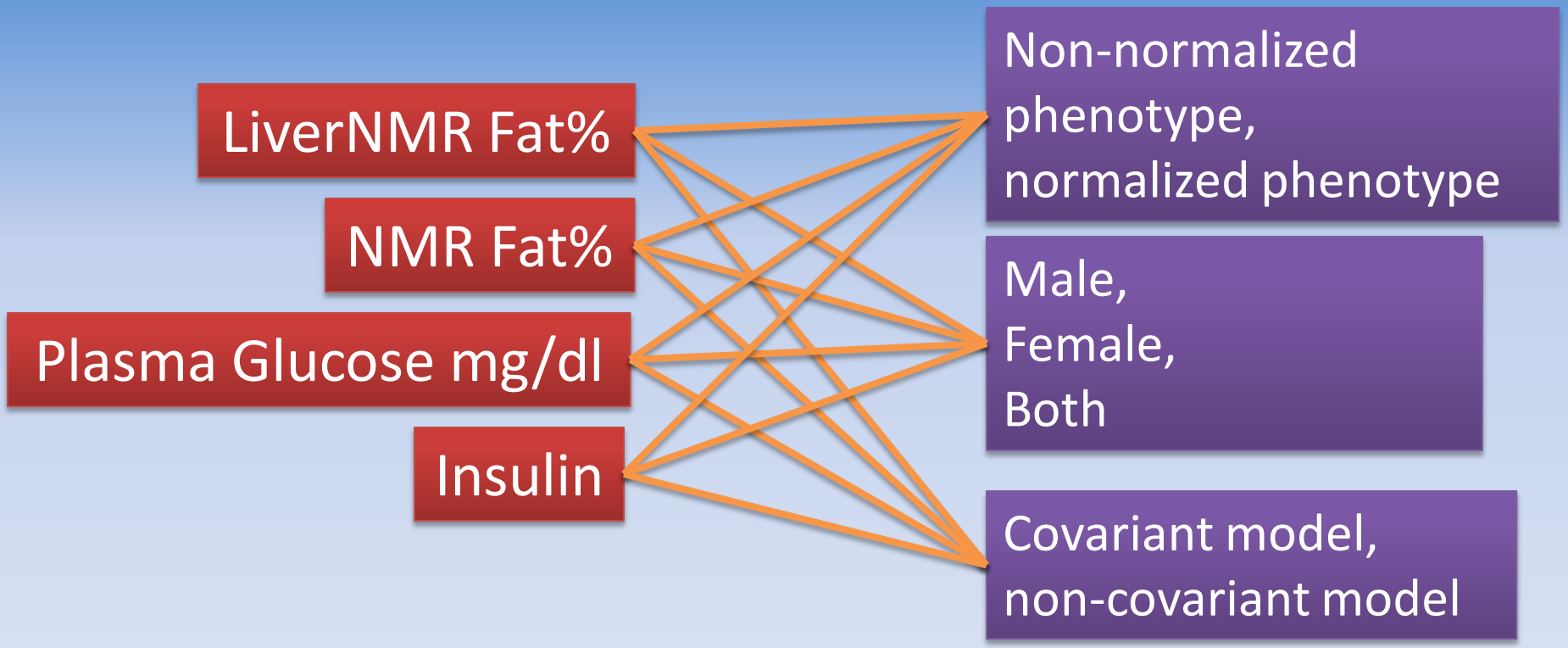

Histogram of bct001(w4_pheno$GCP.2, -0.5)
Histogram of bct transformed data
Frequency
bct001(w4_pheno$GCP.2, -0.5)



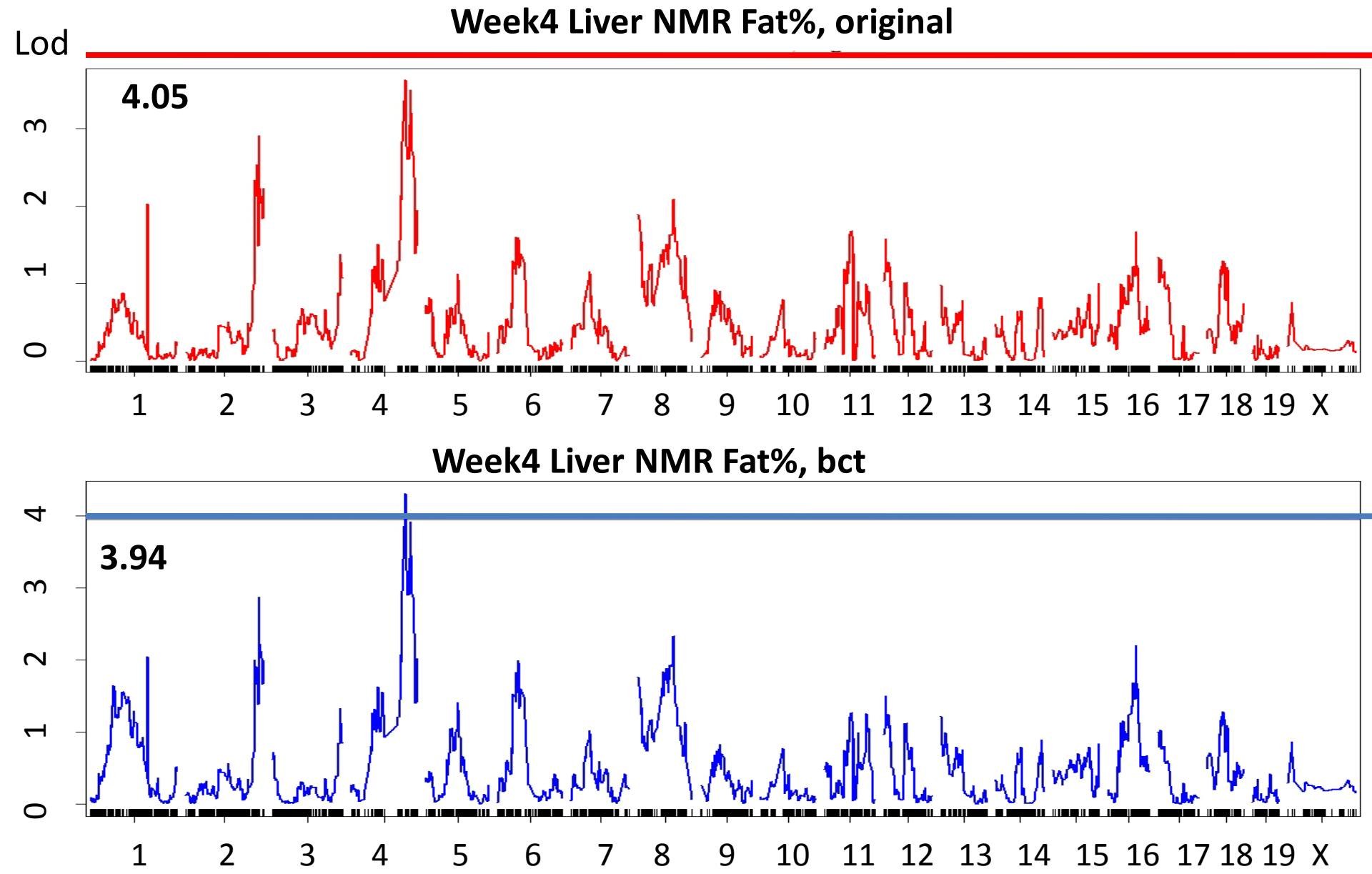


- Advantage of Box-Cox Transformation:
  - The parameter λ can be adjusted to satisfy normal distribution.
  - Negative y could be allowed when adding some modifications.

QTL Mapping

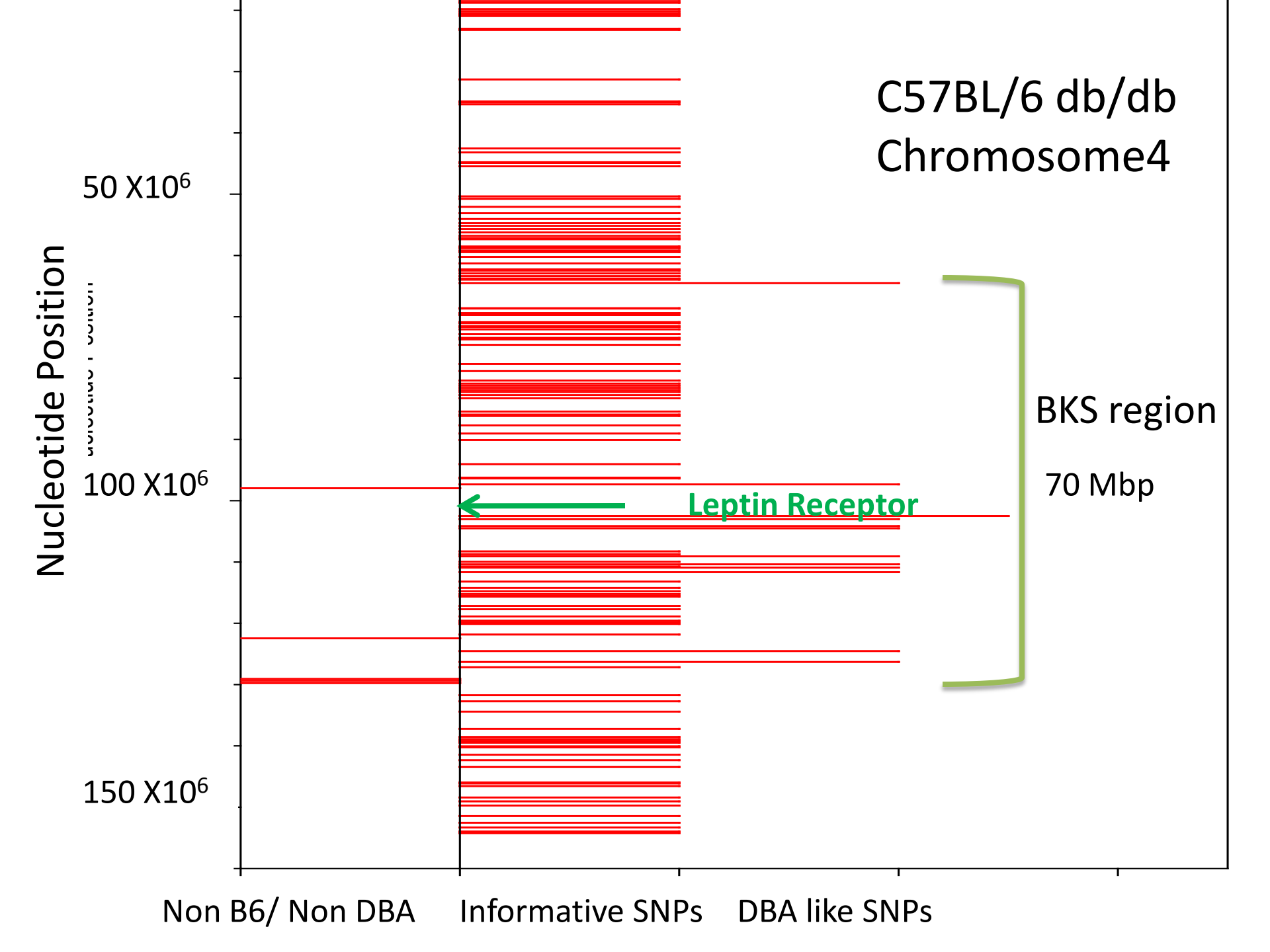

LiverNMR Fat%
NMR Fat%
Plasma Glucose mg/dl
Insulin
Non-normalized phenotype, normalized phenotype
Male, Female, Both
Covariant model, non-covariant model

Normalized and non-normalized data

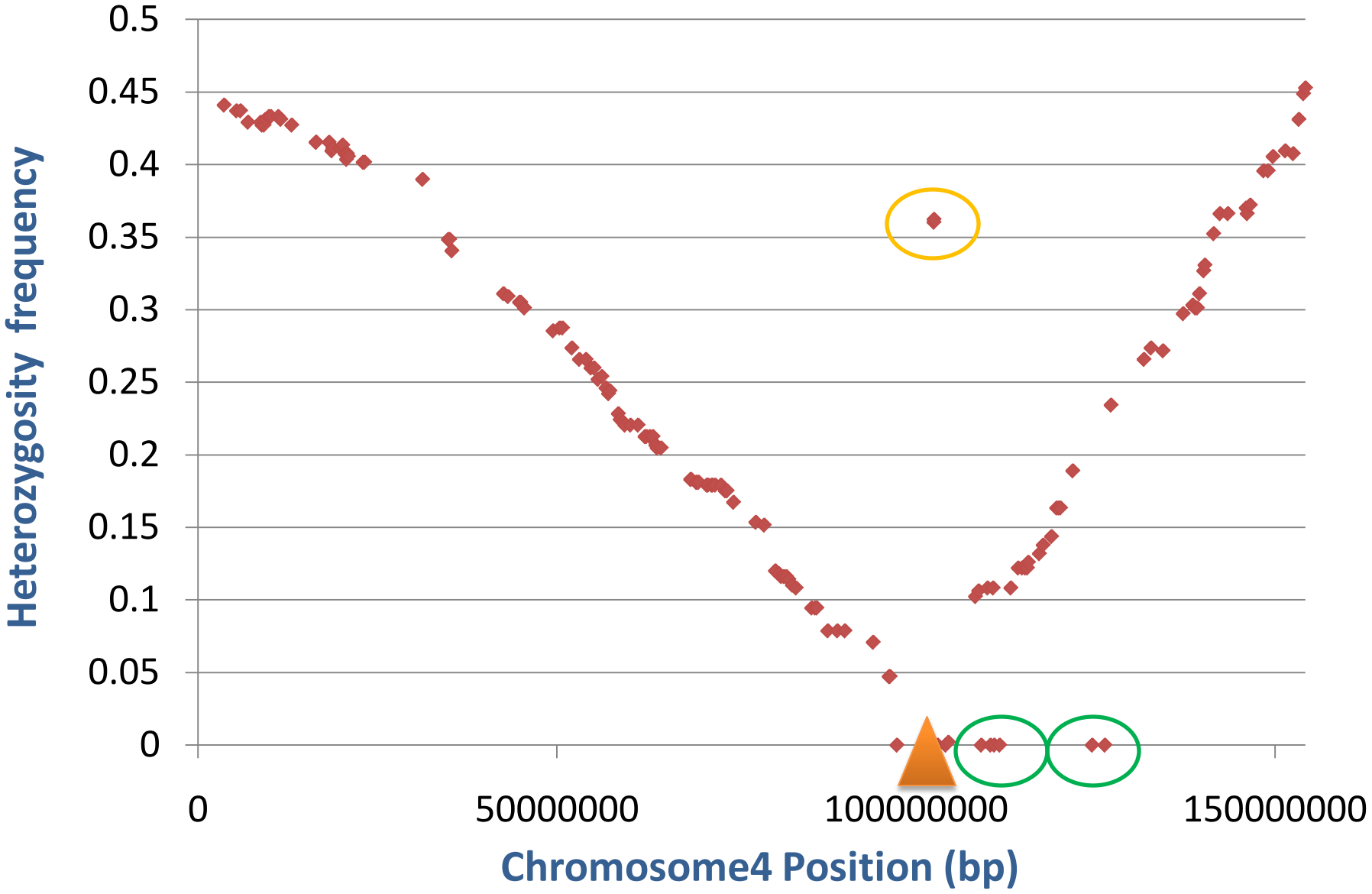


Questions on Leptin Receptor mutation

- What is the origin of Leptin Receptor mutation locus? Where is the locus?
- Does the genotypes on Chromosome 4 Leptin Receptor locus accords with our expectation?
- Does the selection of Leptin Receptor knockout mice (db/db mice) influence the mapping?


C57BL/6 db/db
Chromosome4
Nucleotide Position
50 X10^6
100 X10^6
150 X10^6
BKS region
70 Mbp
Leptin Receptor
Non B6/ Non DBA
Informative SNPs
DBA like SNPs

Chr4 Heterozygosity Plot



Genotypes of two B6-db controls

	B6-db control 1	B6-db control 2
rs13477881	2	0
rs13477882	2	0

0: homozygous allele1
1: heterozygous alleles
2: homozygous allele2

Compare QTLs with previous studies

- For genomewide significant markers (FDR=0.05), drop 1 LOD unit to form the interval.
- For the same trait, combine QTL regions that have the same peak marker.
- Compare the QTL region with previous genomewide scans.
  - Traits: Glucose, insulin, glucose-tolerance (IPGTT).
  - Number of Markers: 69-400

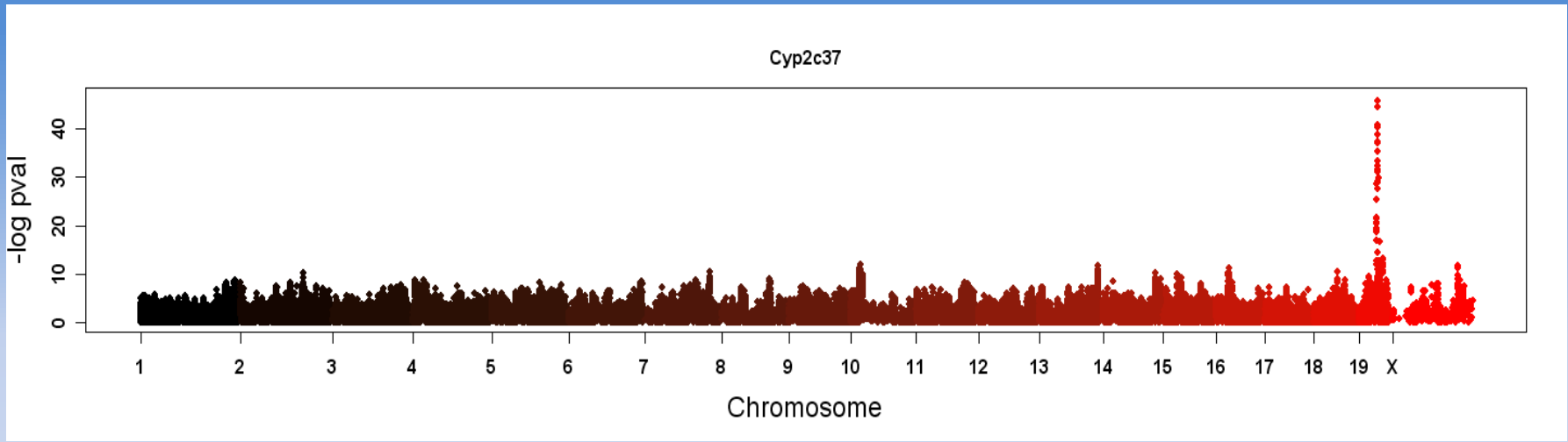
QTL/ Peak Marker	rs13477972	rs13478027	D4Mit203	Nidd/SJL	D4Mit203	Insq1	Nidd12/of_ (Rat)
Peak	127.93 Mbp	141.81 Mbp	124.90 Mbp	114.55 Mbp	129.01 Mb	128.19 Mbp	130.80 Mbp
Markers in Study	2642	2642	227	92	171	630	382
Cross	C57BL/6J x DBA/2	C57BL/6J x DBA/2	C57BL/KsJ x DBA/2, F2	(NZO x SJL) x NZO,	TSOD x BALB/cA , F2	Du6i x DBA/2, F2	OLETF x F344, F2
Trait	Liver Fat%	Liver Fat%	glucose (fasting)	glucose	IPGTT	insulin	IPGTT (fasting; 12h)
Animals in study	252 male&female, 4 Weeks	252 male&female, 12 Weeks	634 female, 9 Weeks	207 male&female, 22 Weeks	144 male, 48 weeks	411 male&female, 6 weeks	160 male, 30 weeks
LOD score	4.31	4.11	4.9	3.6	3.3	3.6	4.0

Conclusion

- In week12, some mice are diabetic, some are insulin resistant, and some are normal.
- The db locus on Chr4 has BKS origin. It influences the genotype on chr4, and it influences qtl mapping.
- Summary QTLs of LiverNMR Fat%, BodyNMR Fat%, Plasma Glucose and Insulin:

LiverNMR Fat%	BodyNMR Fat%	Plasma Glucose	Insulin
Chr4: 120Mb-141Mb, 129Mb-149Mb	Chr11:101Mb-106Mb, 87Mb-119Mb; Chr5: 12.8Mb-18.7Mb	Chr5: 114Mb-147Mb; 106Mb-147Mb	Chr5: 119Mb-147Mb, 135Mb-147Mb, 142Mb-147Mb; Chr2: 91Mb-123MB

Genome wide association in a panel of 100 mouse strains



Comparison with same eQTL in F2 cross