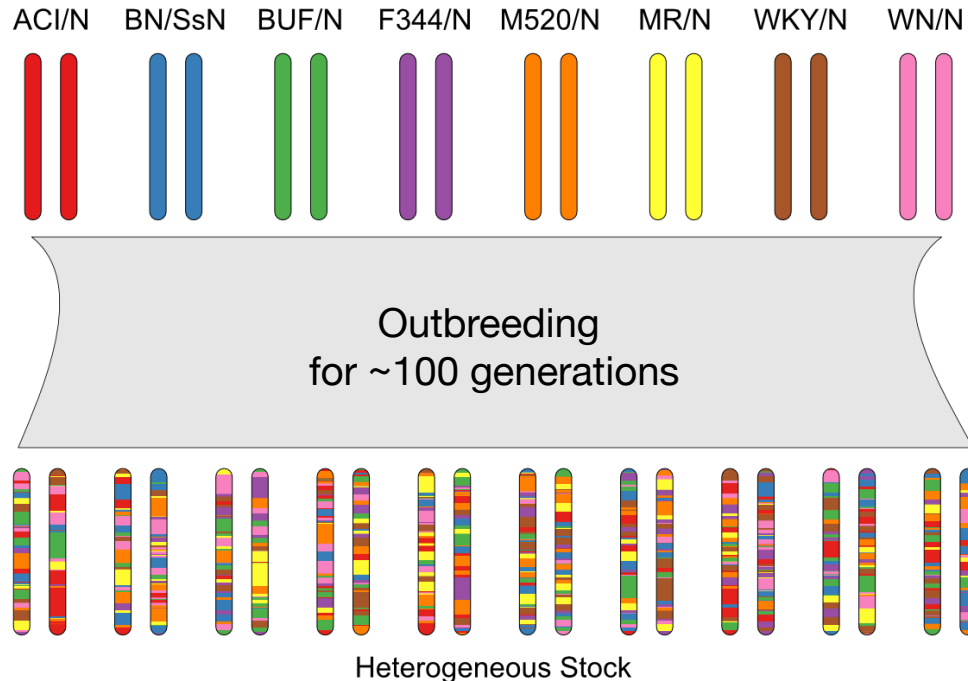
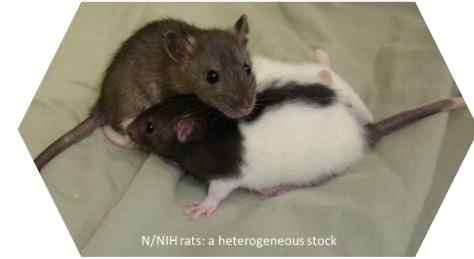


# Genome-wide association of adiposity in Heterogeneous Stock rats

**Apurva S. Chitre**

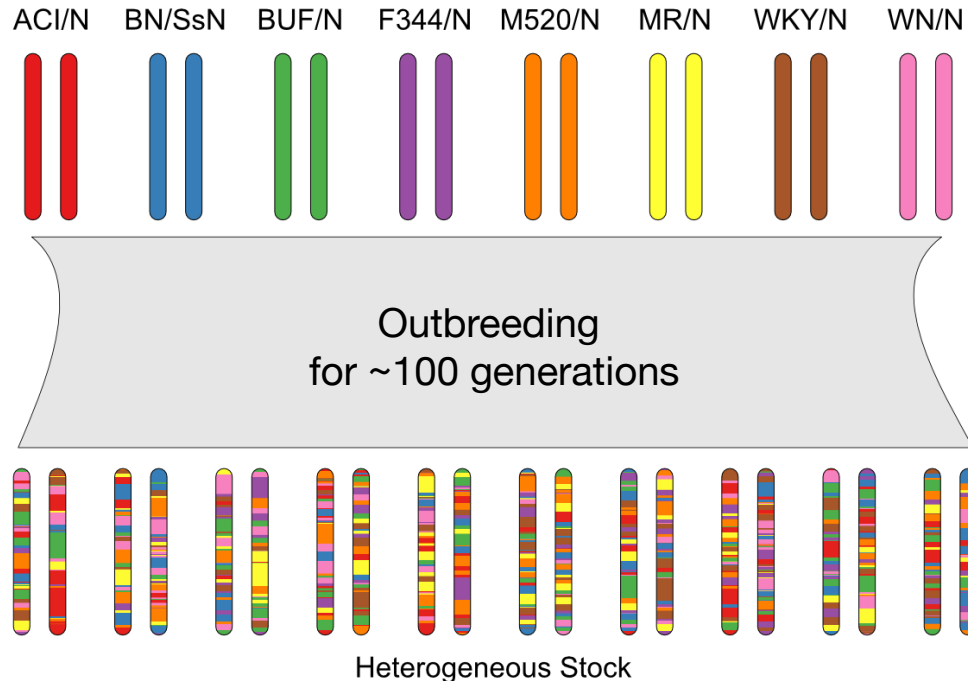
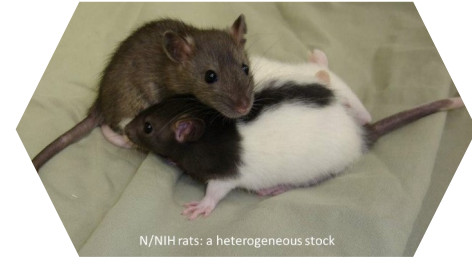
**PhD student in Bioinformatics and Systems Biology program  
Palmer Lab, UC San Diego**

# Utilizing Heterogeneous Stock Rats for Genetic Mapping



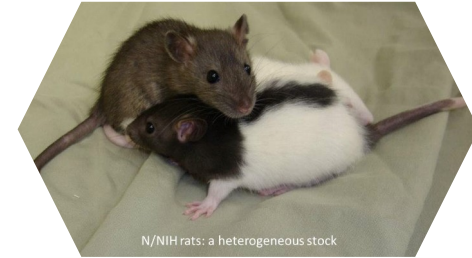
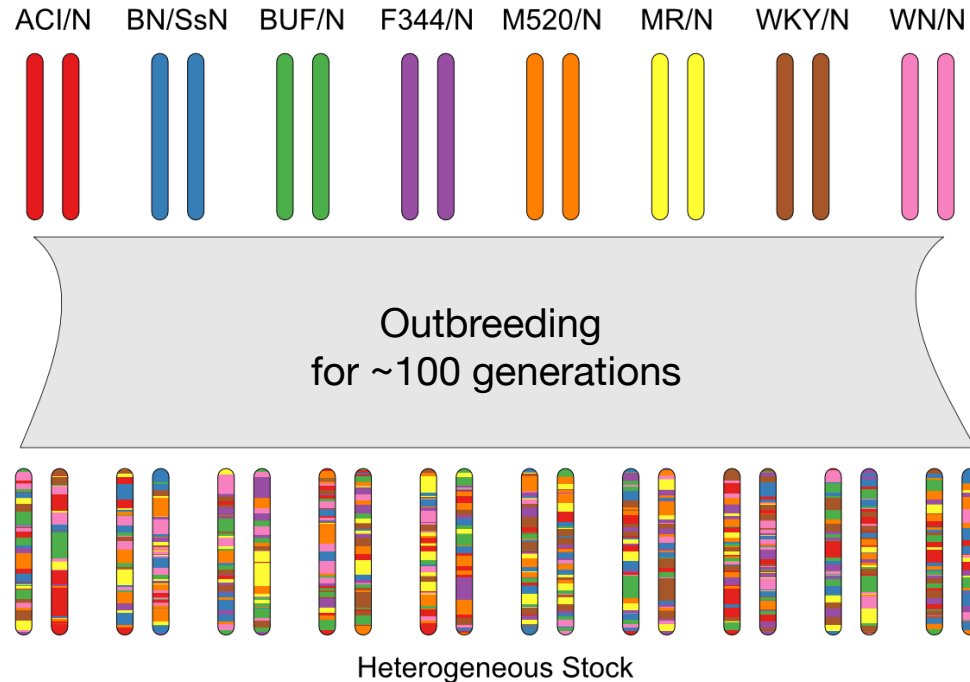
In 1984, at the NIH, 8 inbred rat strains were interbred to develop an outbred population enriched with alleles from these diverse founders.

# Utilizing Heterogeneous Stock Rats for Genetic Mapping



- In this population, at any given locus, a given individual might have inherited their alleles from different strains, contributing to a diverse genetic makeup.
- Each animal is genetically and phenotypically distinct.

# Heterogenous stock (HS) rats



Dr. Leah Solberg Woods



**Wake Forest University**  
School of Medicine

Aiming to link genotype and phenotype  
through GWAS in HS rats



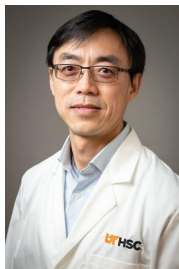
Genes and Addiction  
**NIDA Center for GWAS in Outbred Rats**

- Lead collaborative efforts in studying genetics of drug abuse in HS rats.
- Distributes HS rats to various research groups for targeted phenotyping.
- Conducts genotyping and GWAS.



# Genes and Addiction

## NIDA Center for GWAS in Outbred Rats



Hao Chen

Socially-acquired  
nicotine self-  
administration

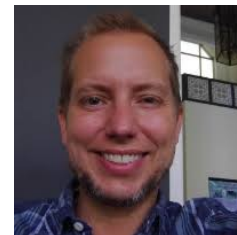


Shelly Flagel

Genetic Studies of  
Incentive Saliency



David Dietz



Paul Meyer

Association between behavioral  
regulation and cocaine cue  
preference

# Post-Behavioral Phenotyping Trait Measurements



- **Fasting Period:**  $17 \pm 2$  hours
- **Measured Traits:**
  - Body Weight
  - Body Length (with/without tail)
  - Fasting Glucose Levels
- **Visceral Fat Pads:**
  - Retroperitoneal Fat
  - Epididymal Fat (Males)
  - Parametrial Fat (Females)



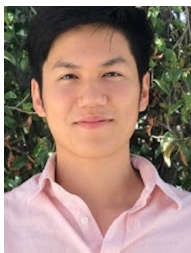
# Study Scale and Demographics

Center	Age at Euthanasia	Sample Size	Housing	Drug Treatment	Drug Dose	Diet
University of Tennessee - Experimental	73 +/- 12 days	2,766	Pairs	Nicotine	Variable (self-administration)	Teklad LM-485 Mouse/Rat Diet
University of Tennessee - Breeders	169 +/- 34 days	1,032	-	-	-	
University at Buffalo - Dietz lab	198 +/- 13 days	2,726	Pairs	Cocaine	10 mg/kg	Envigo Teklad 18% Protein Rodent Diet
University at Buffalo - Meyer lab	164 +/- 14 days	707	Pairs	-	-	
University of Michigan	89 +/- 6 days	1,626	Trios	Cocaine	15 mg/kg	Labdiet Picolab Rodent Diet Irradiated

Total sample size = 8,857 male and female Heterogenous Stock rats

# Genotyping

- Spleen tissue from the phenotyped rats were shipped to the Palmer lab at UC San Diego.
- Through low-coverage whole-genome sequencing (lcWGS), we accurately identified 7.32 million SNPs with a 99.8% accuracy rate.



Khai Nguyen



Clara  
Ortez



Yizhi  
Wang



Benjamin  
Johnson



Denghui  
Chen

# GWAS pipeline

- Phenotyping center differences accounted for as covariates, mitigating site-specific variations such as diet and housing.
- SNP heritability ( $h^2$ ) estimated with GCTA-GREML.
- Genetic correlations via bivariate GREML (GCTA).
- GWAS employed GCTA's linear mixed model with a GRM to account for complex HS population relationships.
- Significance thresholds established through permutation.



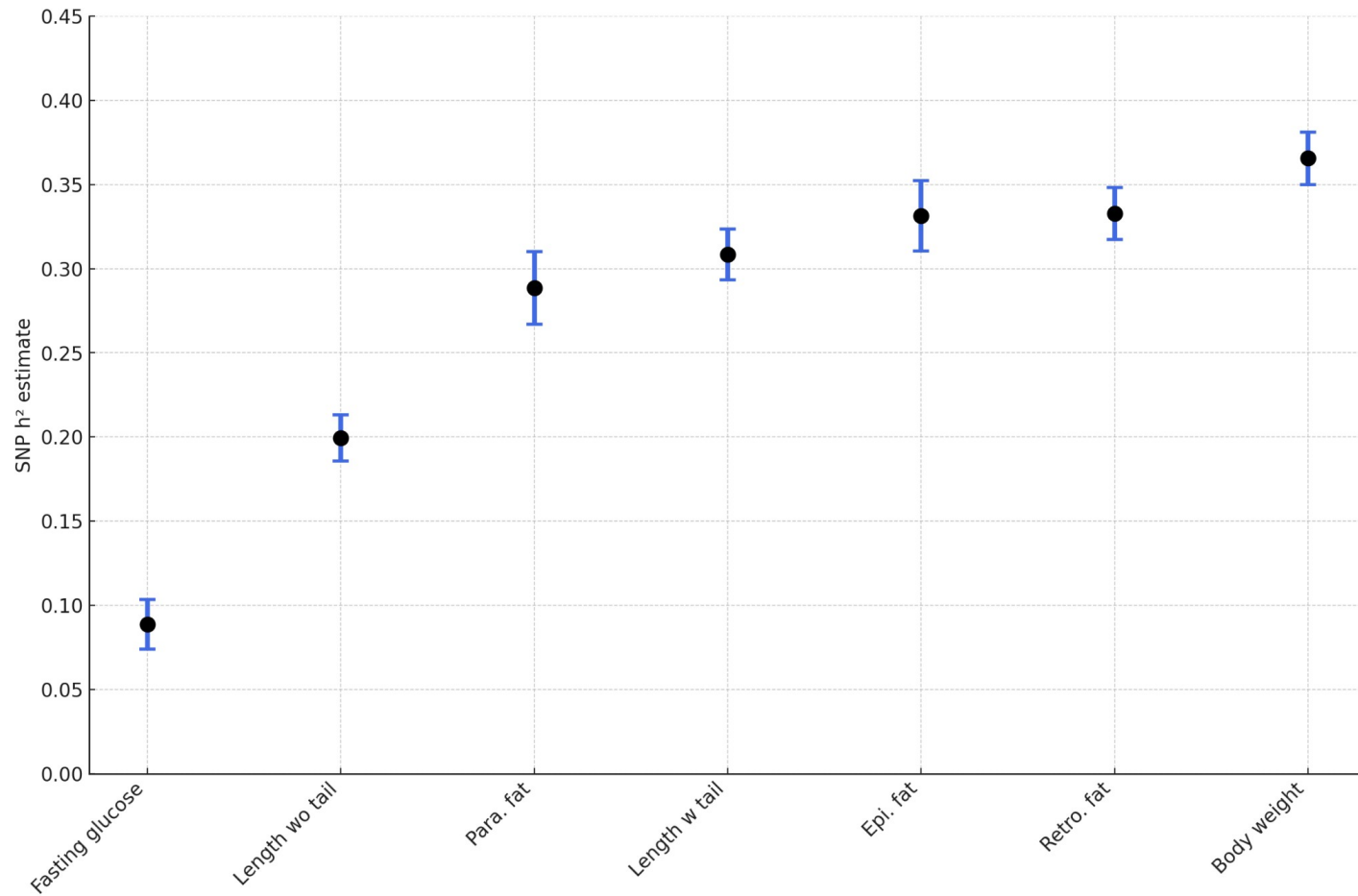
Thiago  
Sanches



Apurva  
Chitre

Are the adiposity traits heritable?

# Adiposity traits exhibit high SNP heritability



# Phenotypic and Genetic Correlation

## **Phenotypic Correlation:**

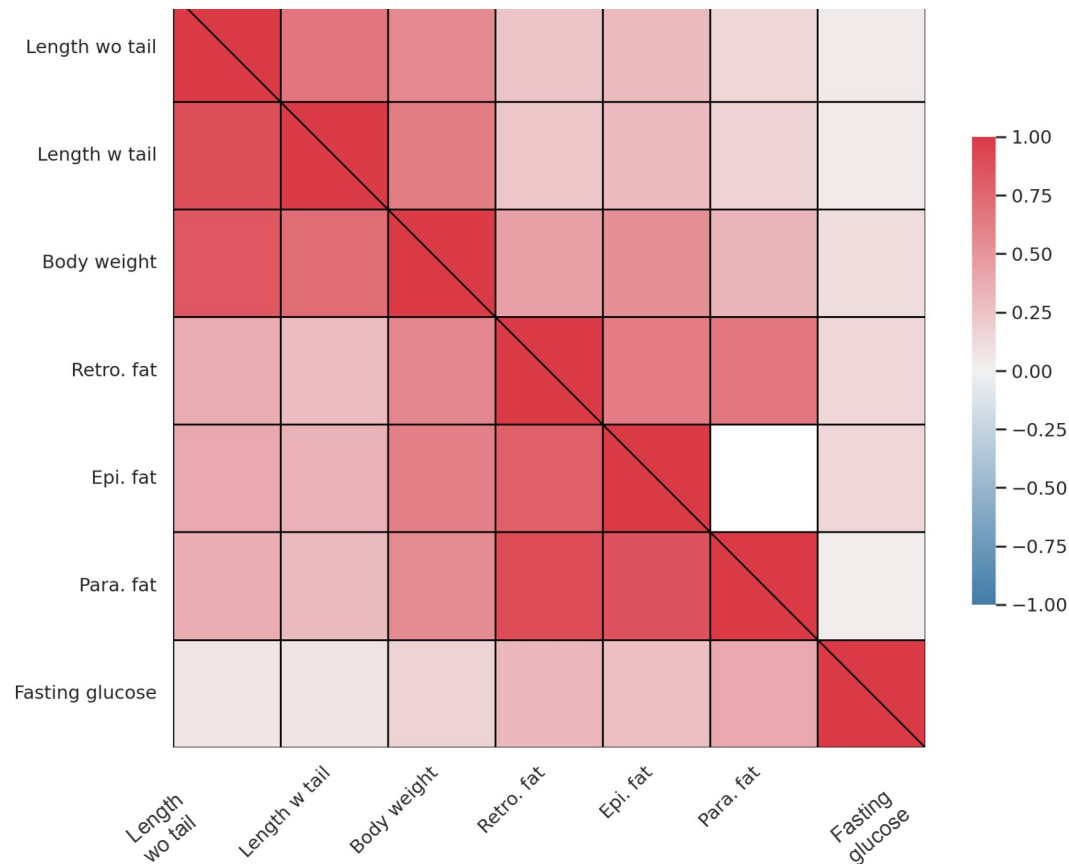
- How one phenotype relates to another in observed values.

## **Genetic Correlation:**

- Shared variance between two traits due to genetics.
- Can be estimated even if traits aren't measured in the same individuals.

# Genetic and phenotypic correlations

Upper triangle displays phenotypic correlations, while the lower triangle shows genetic correlations.





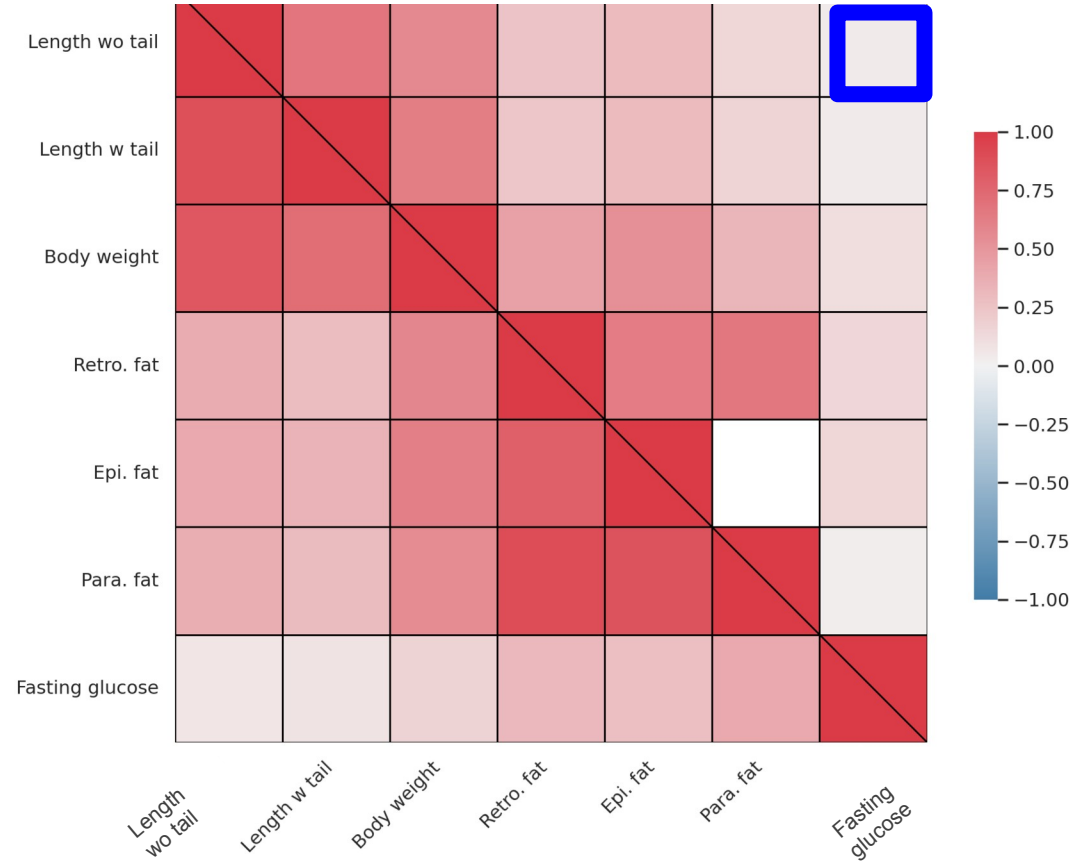


# Genetic and phenotypic correlations

Upper triangle displays phenotypic correlations, while the lower triangle shows genetic correlations.

## Phenotypic correlations:

- Strongest estimate was between length with tail and length without tail.
- Weakest phenotypic correlation was between parametrial fat weight and fasting glucose.

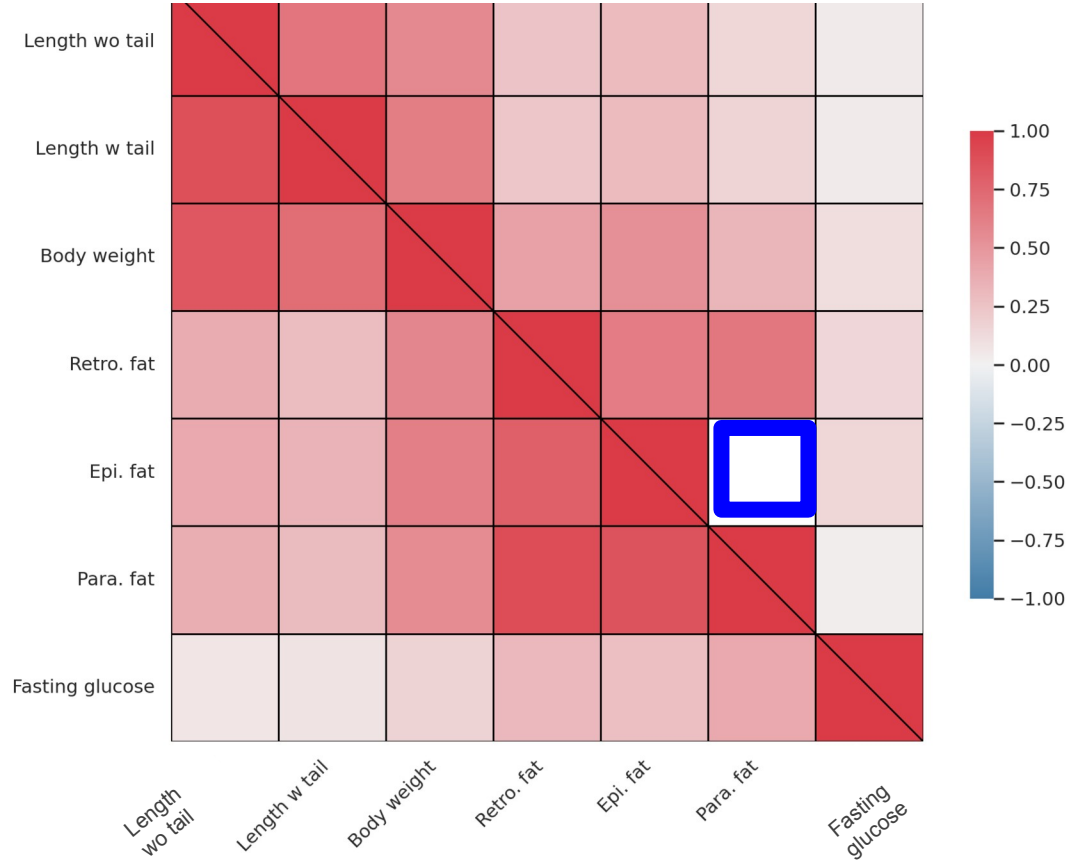


## Genetic and phenotypic correlations

Upper triangle displays phenotypic correlations, while the lower triangle shows genetic correlations.

## Phenotypic correlations:

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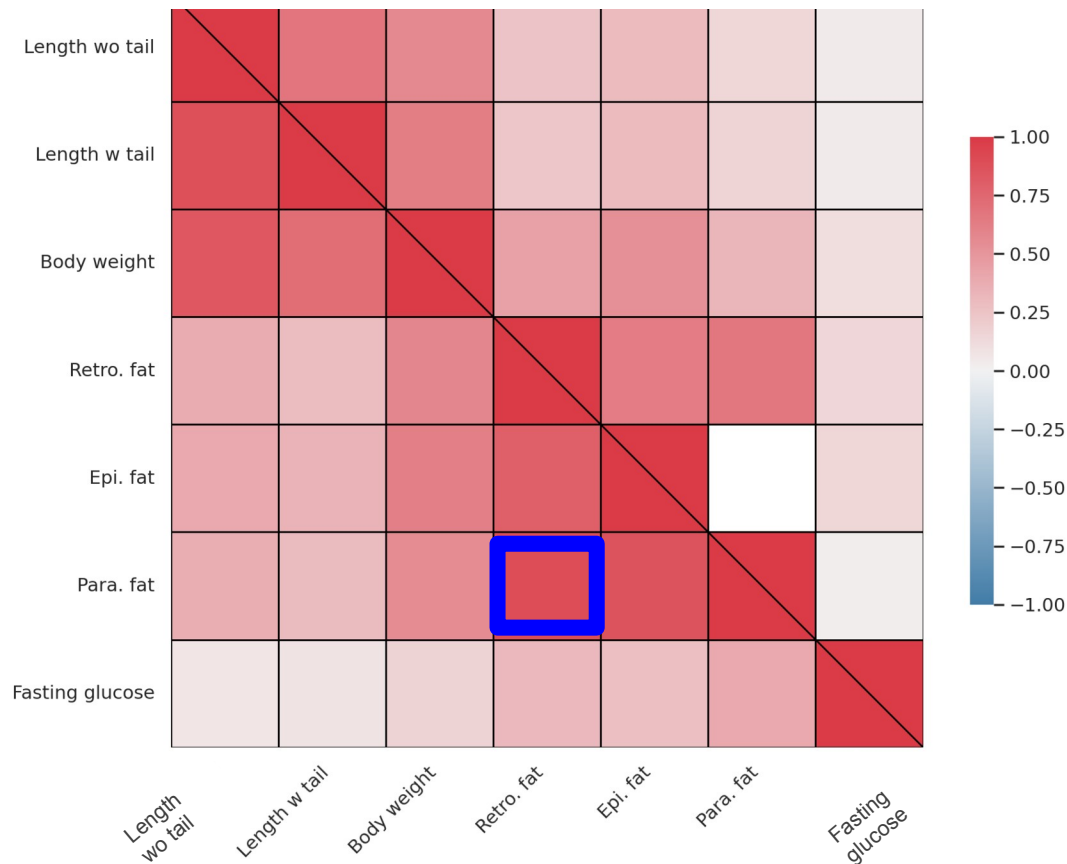


# Genetic and phenotypic correlations

Upper triangle displays phenotypic correlations, while the lower triangle shows genetic correlations.

## Genetic correlations:

- Highest genetic correlation observed was between parametrial fat weight and retroperitoneal fat weight.
- Lowest genetic correlation was between length without tail and fasting glucose.

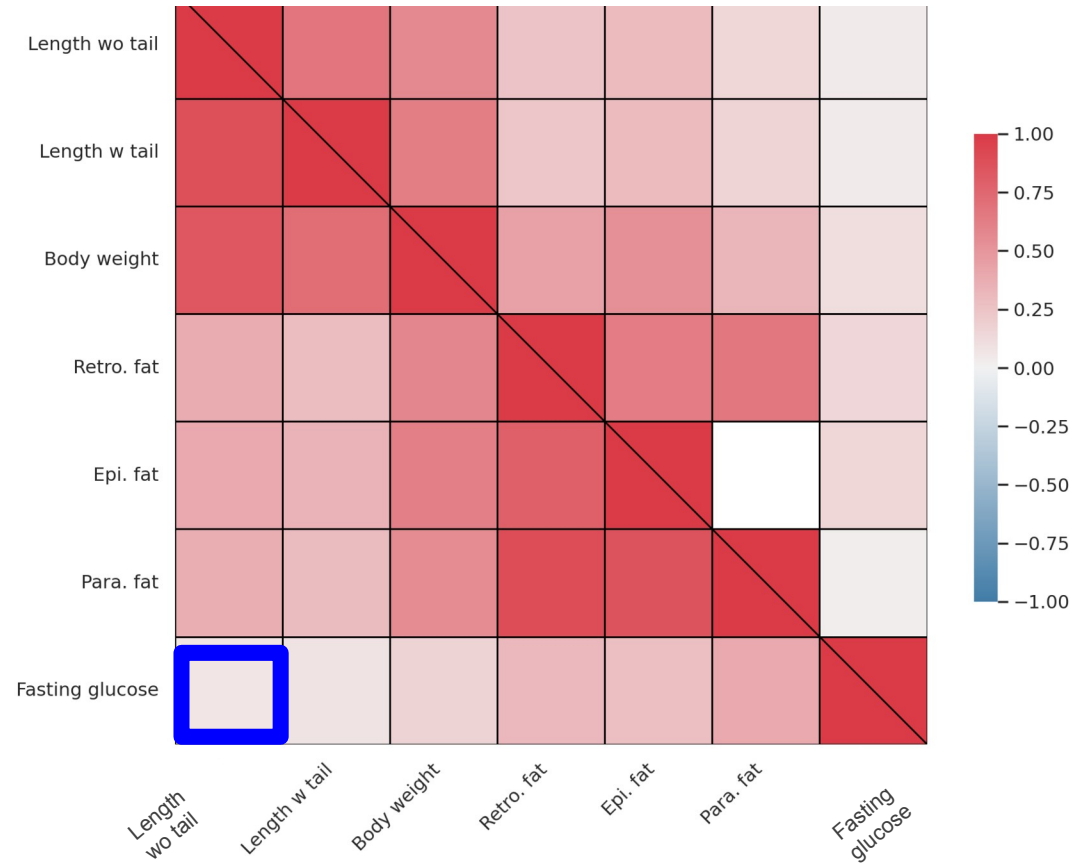


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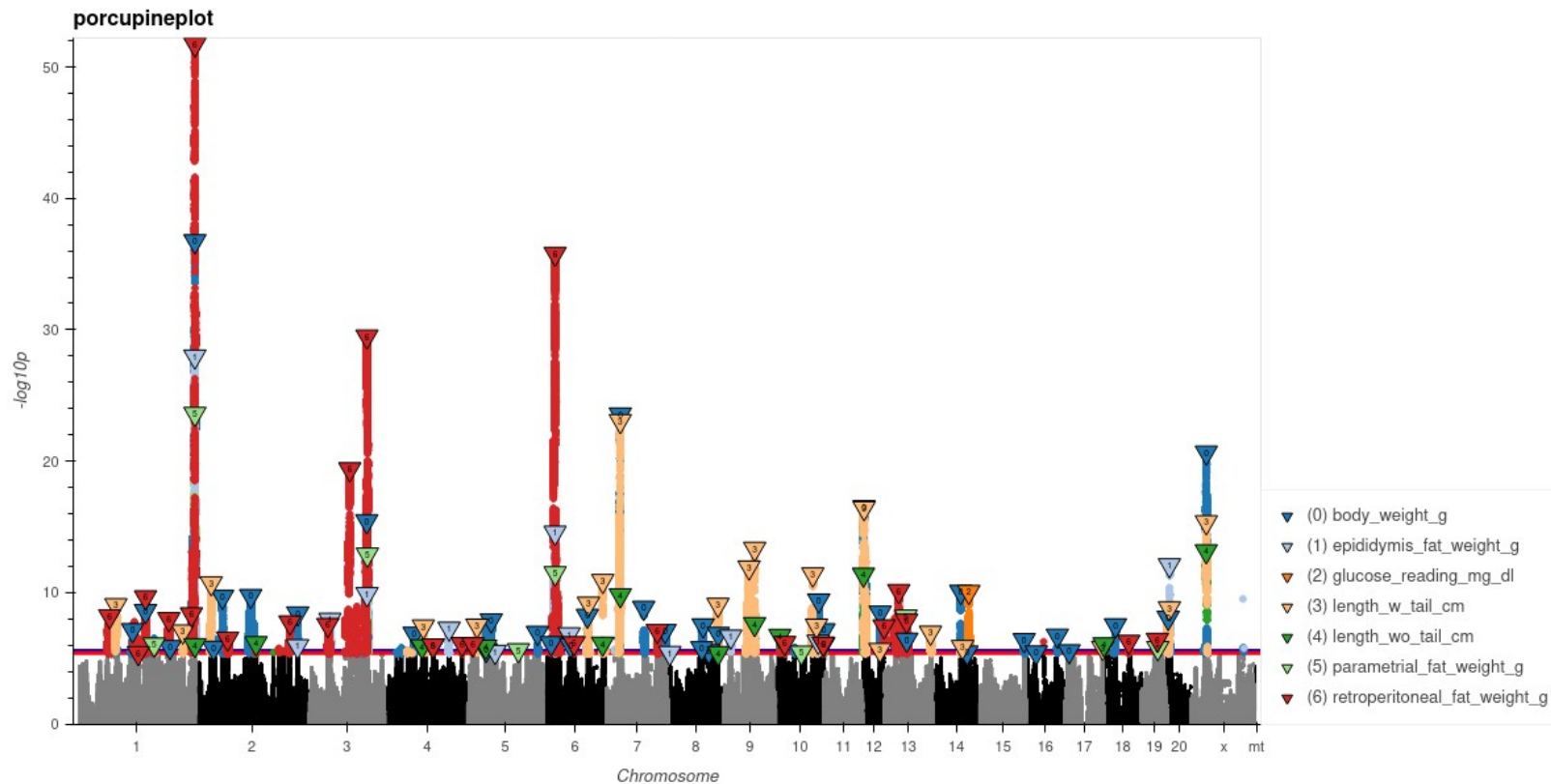
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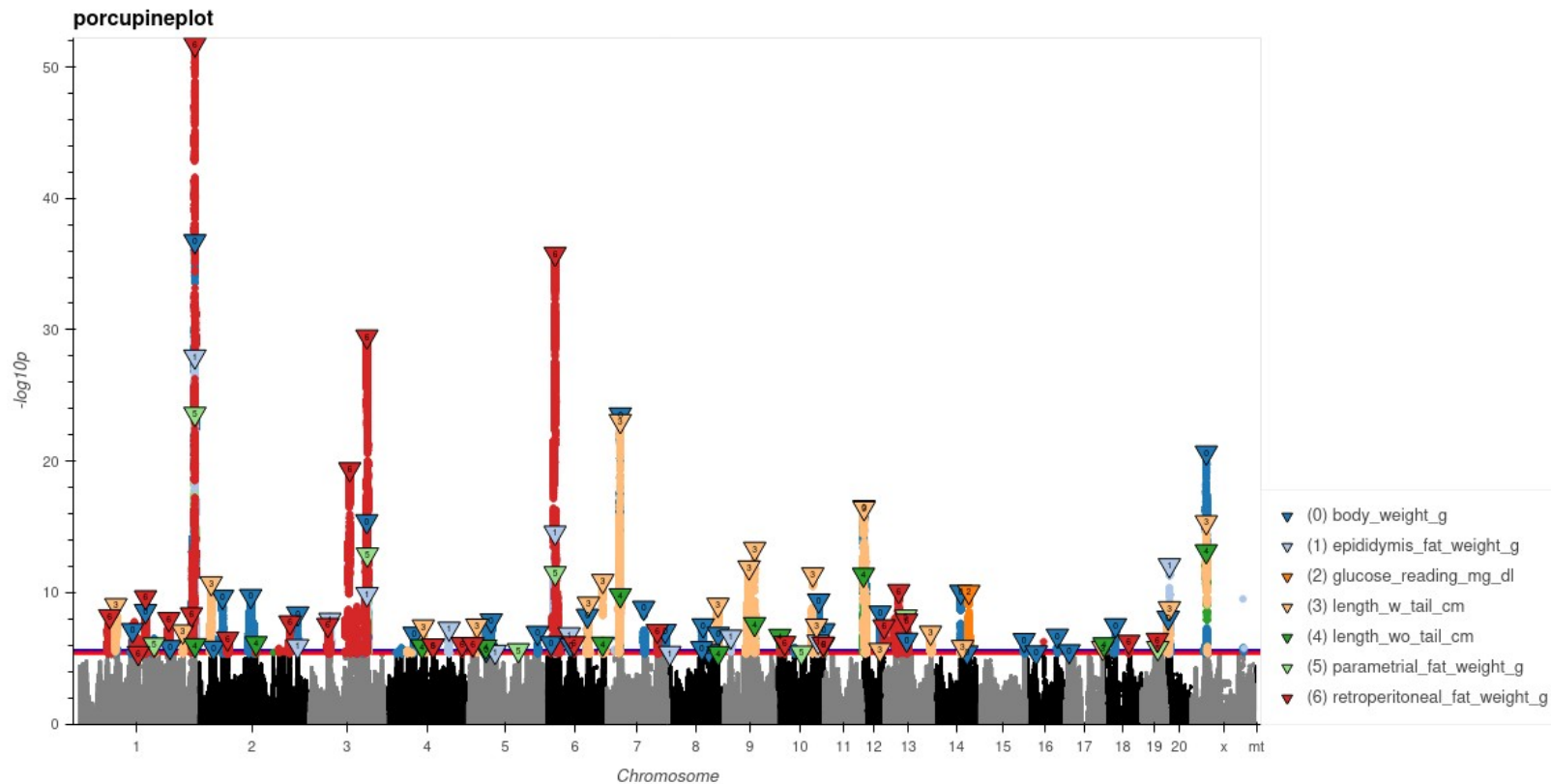


Are there specific chromosomal locations associated with adiposity traits in our HS rat population?

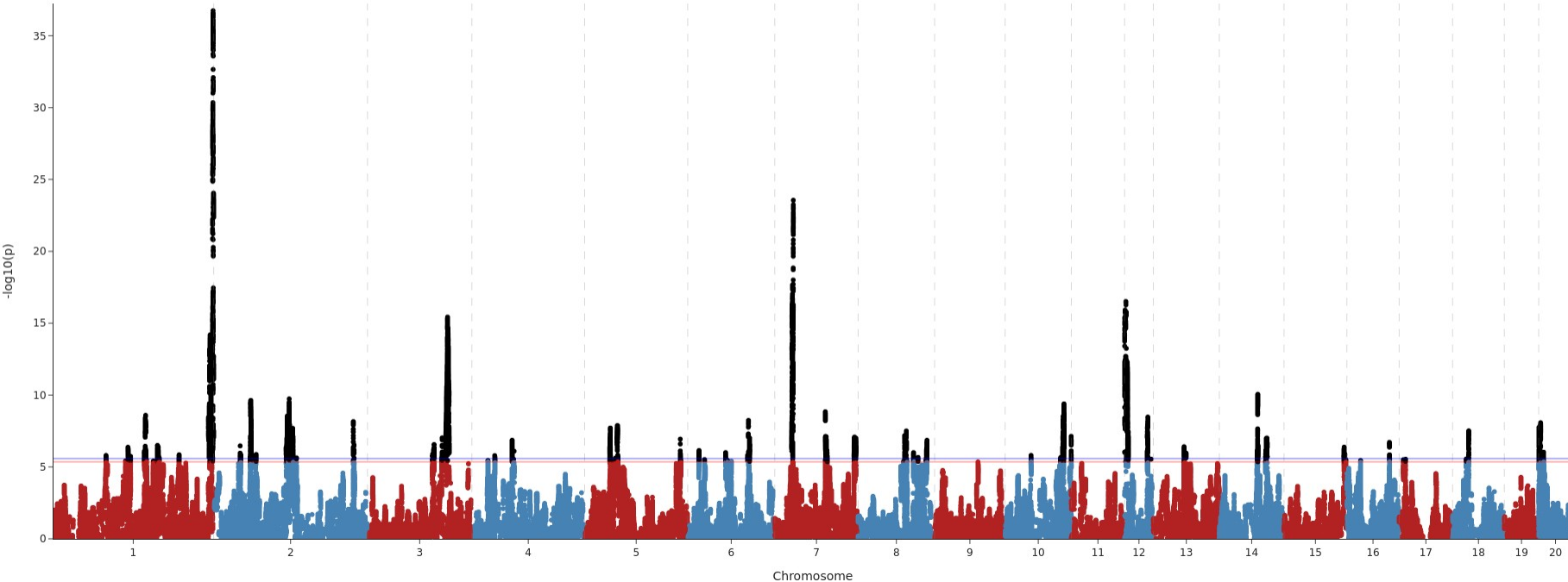
# Identification of multiple GWAS hits, N= 8,857



# Identification of 116 independent QTLs for 7 adiposity traits



37 independent QTLs for Body weight, N= 8,857  
SNP heritability:  $0.37 \pm 0.02$



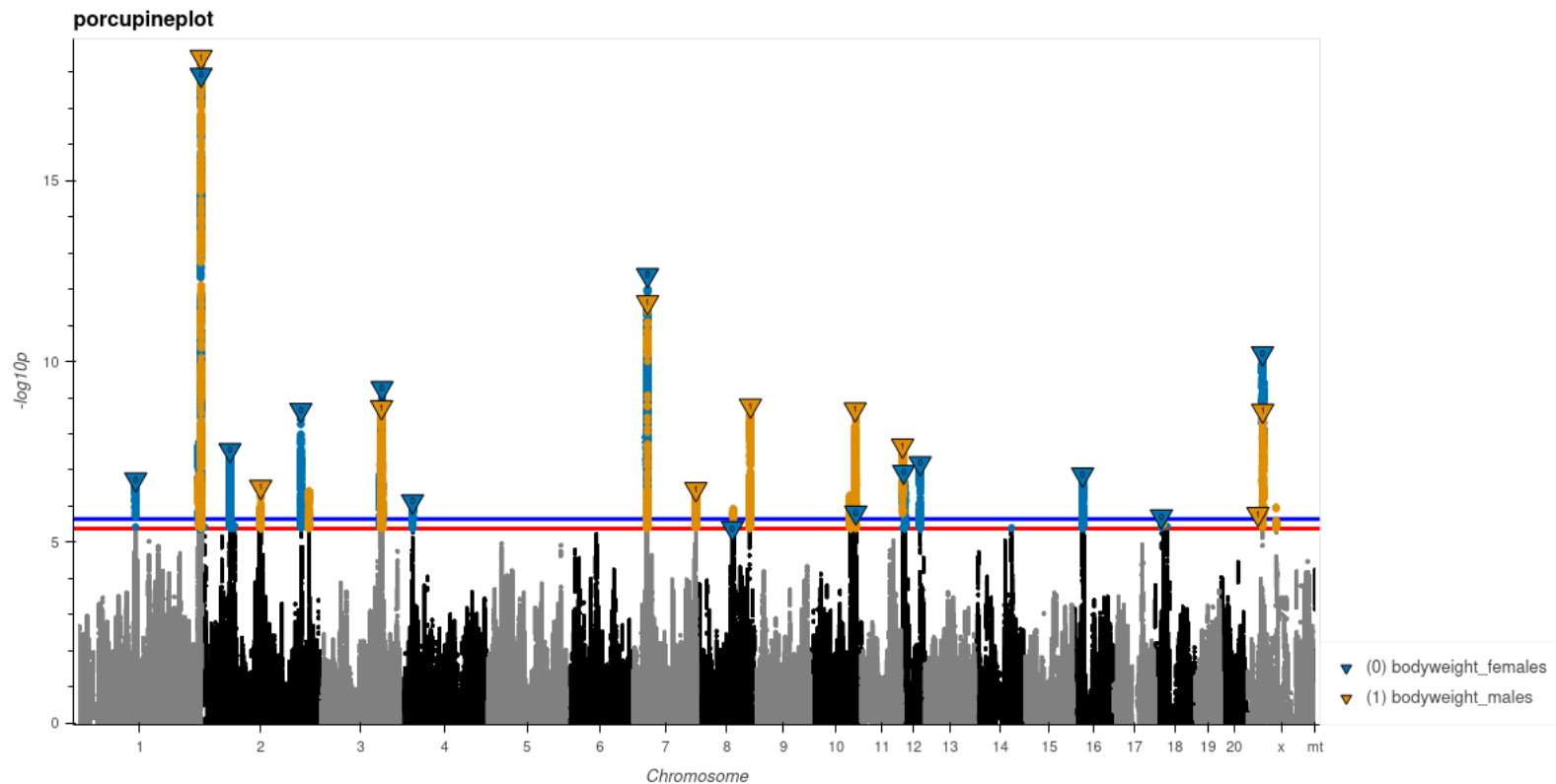


Are Body Weight QTLs Sex-Specific?

# Sex-stratified GWAS analysis for body weight

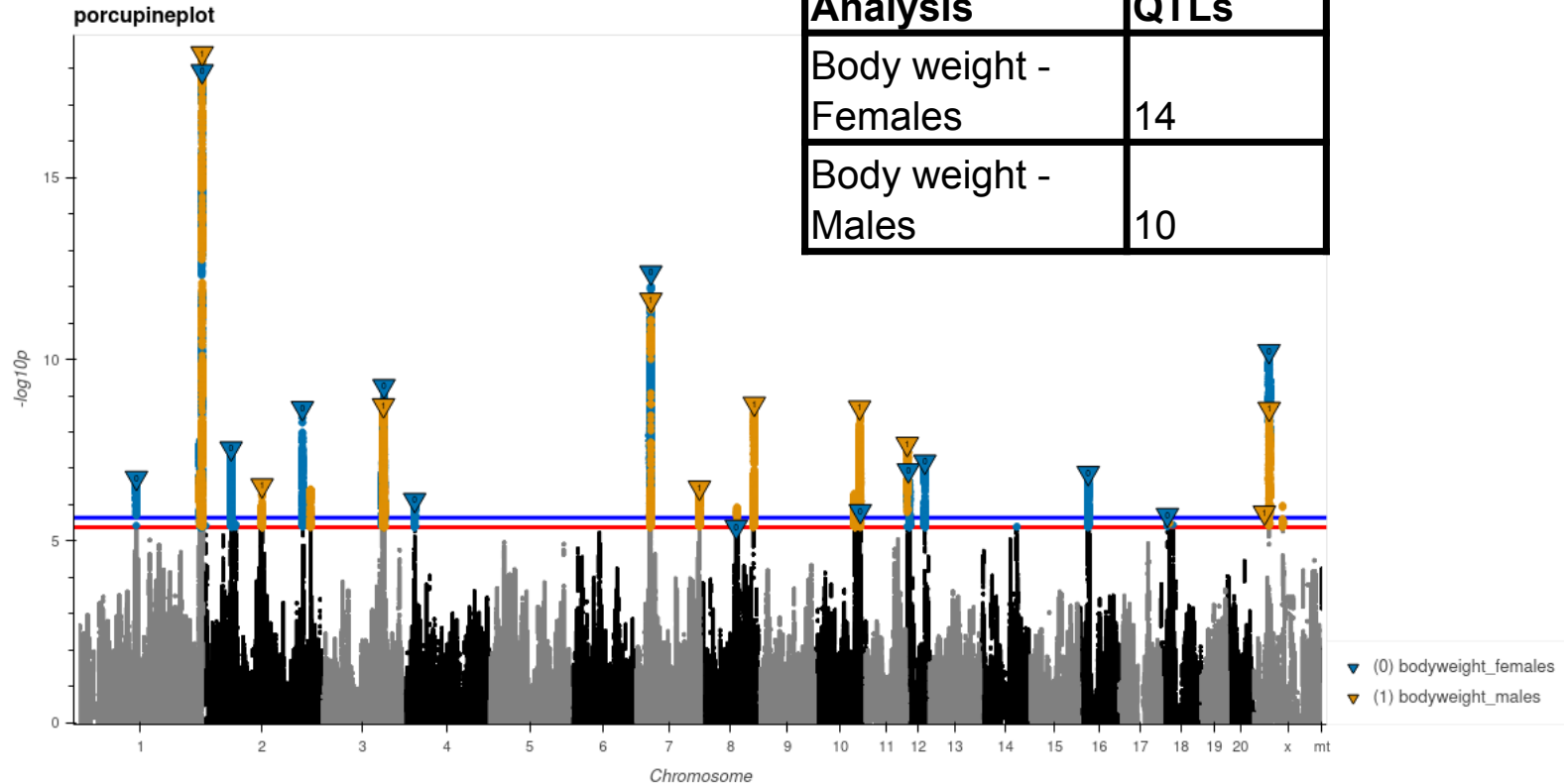
- Dataset division :
  - Females:  $n = 4,173$
  - Males:  $n = 4,285$
- Applied standard GWAS pipeline separately to each sex
- Sex was not included as a covariate

# Sex-specific GWAS reveals distinct and shared loci



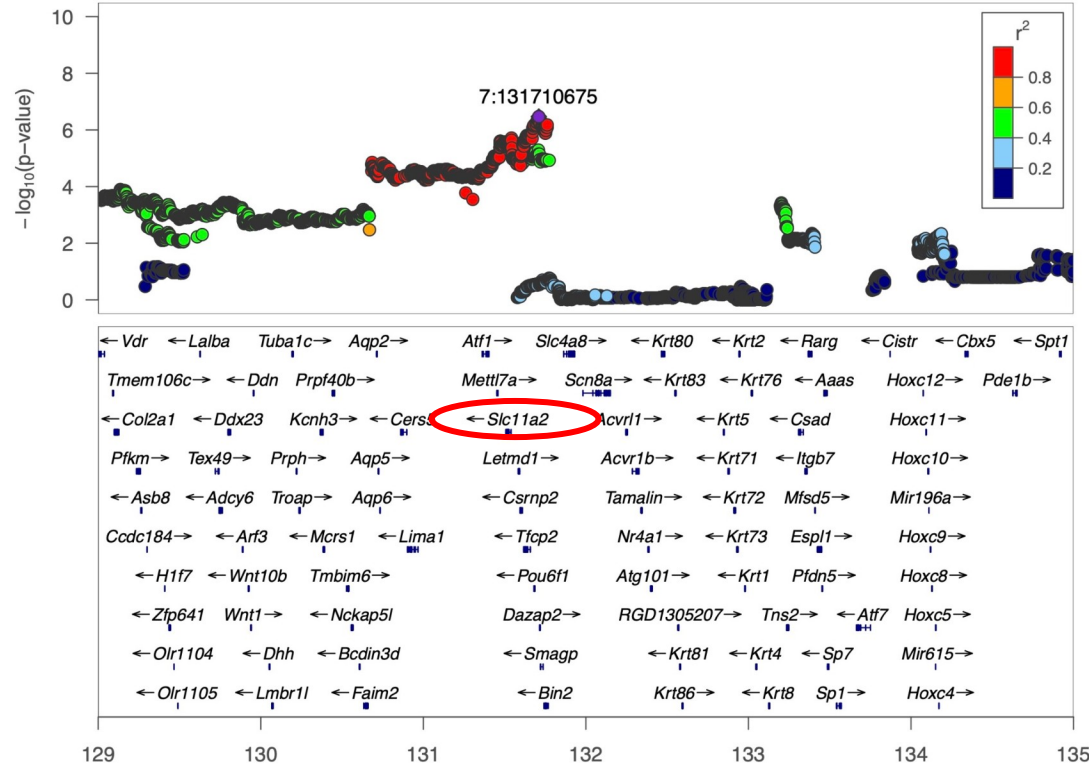
# Sex-specific GWAS reveals distinct and shared loci

Analysis	QTLs
Body weight - Females	14
Body weight - Males	10



# Chr7: 131.71Mb locus specific to males

- Identified a high-impact splice donor variant in gene *Slc11a2* potentially altering mRNA splicing.
- SLC11A2* gene linked to obesity and iron accumulation in skeletal muscle. (González-Domínguez et al. 2020)

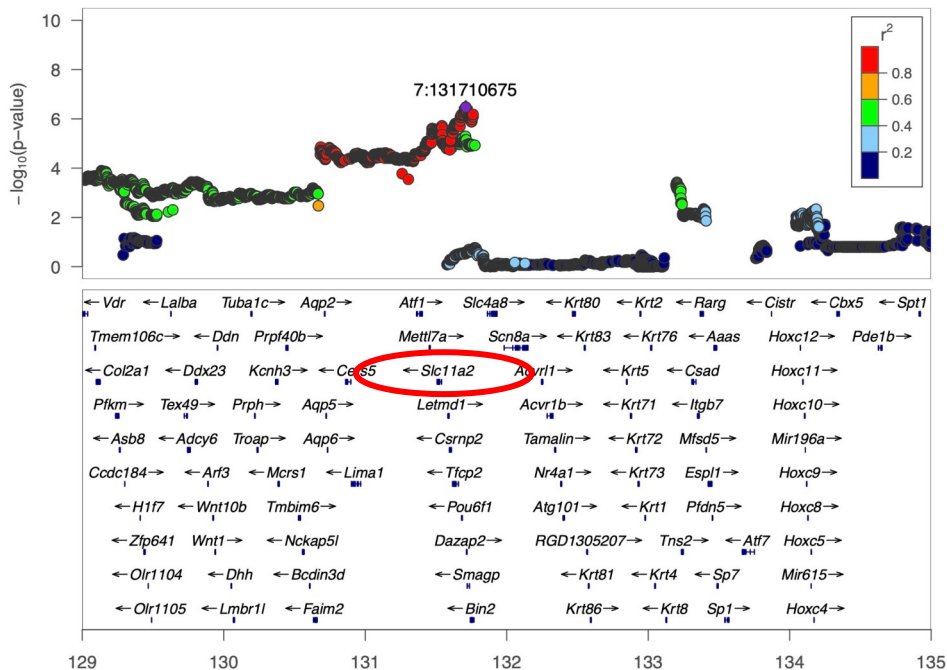


What other traits also show association at this locus?

# Chr7 : 131.71Mb locus specific to males

## Results from PheWAS

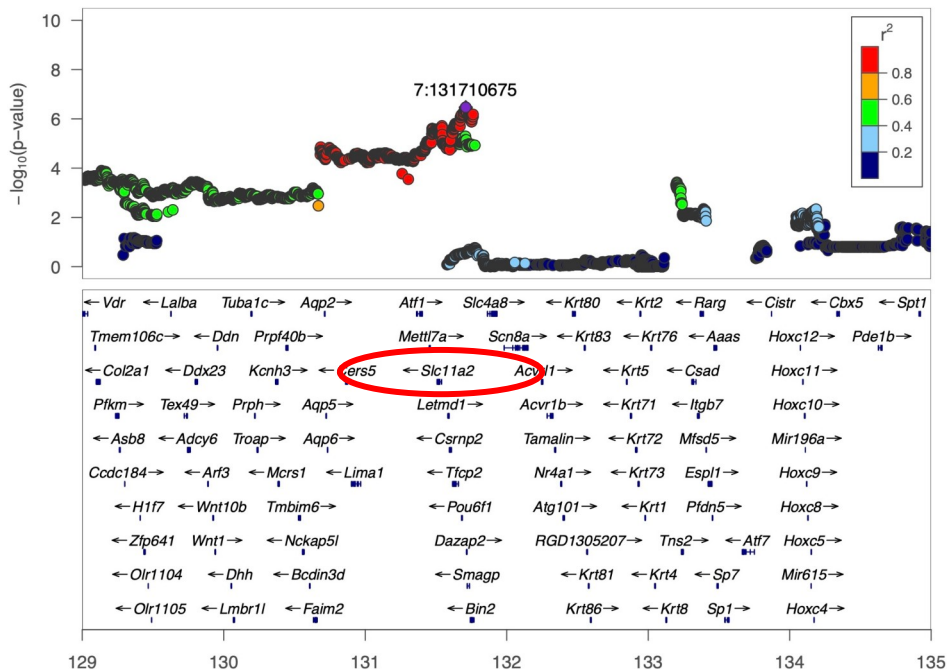
Category	Trait	Significance ( $-\log_{10}$ P-value)
Muscle Phenotypes [Lionikas Lab]	Tibialis Anterior Weight (grams)	4.47
	Extensor Digitorum Longus Weight (grams)	6.105
Bone Phenotypes [Adams Lab]	Bone Stiffness	6.291
	Bone Surface Area	4.391



# Chr7 : 131.71Mb locus specific to males

## Results from PheWAS

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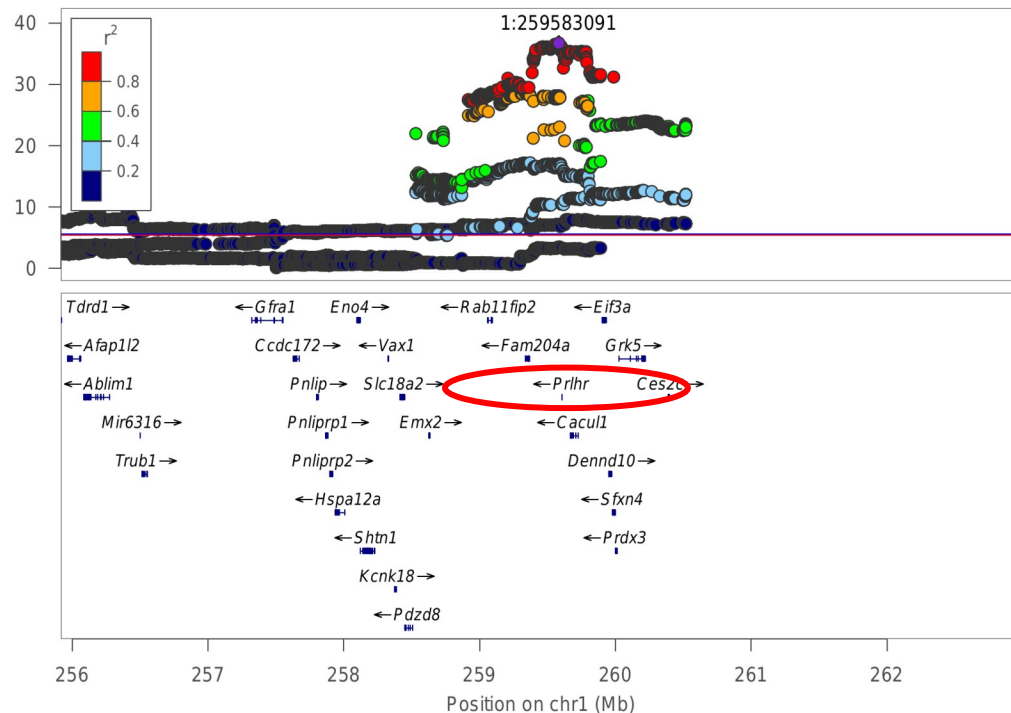
Variation at this locus may influence multiple related traits through common biological pathways



# Shared locus in male and female body weight GWAS

- Within this QTL, we've identified a 'HIGH' impact 'start\_lost' coding variant in *Prhr* gene.
- Loss of start codon likely disrupts protein translation and function

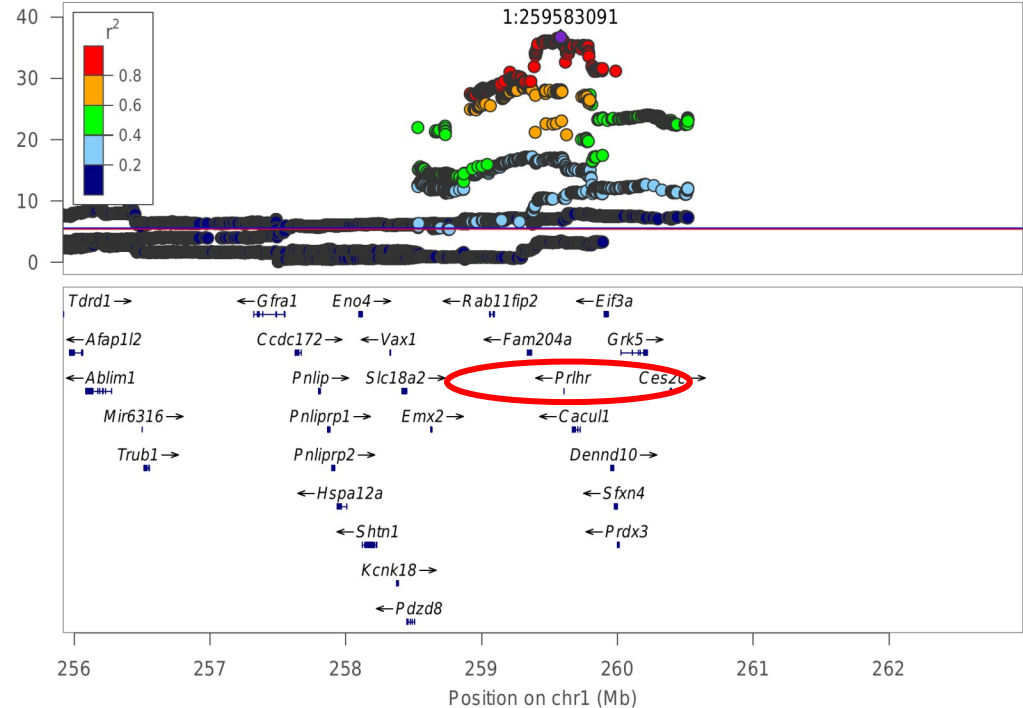
Chr1: 259.61 Mb locus for bodyweight



# Shared locus in male and female body weight GWAS

Chr1: 259.61 Mb locus for bodyweight

- Talbot et al. 2023 identified multiple rare variants in PRLHR (GPR10) linked to severe obesity, impairing ligand binding and G protein signaling, supporting GPR10's role in energy homeostasis and potential as a weight-loss therapeutic target.
- Keele et al.'s 2018 (PMID: 29193816) GWAS on a different HS rat cohort also highlighted this locus, supporting our results.



# Conclusions

- This is the largest GWAS ever conducted in rodents.
- We performed sex stratified GWAS and identified male- and female-specific loci for body weight.
- Some loci found to be common in both males and females.
- We replicated previously identified loci (Keele et. al. 2018) and identified numerous novel loci for multiple adiposity traits.
- This result demonstrates the importance of large sample sizes for genetic dissection of complex traits

# Future directions

- Ongoing phenotyping by partner labs will significantly increase our dataset.
- Anticipate re-analysis with ~1000 additional animals to strengthen our findings.
- Perform sex as an interaction term GWAS.

# Acknowledgements



Genes and Addiction  
NIDA Center for GWAS in Outbred Rats



# References

- Keele GR, et al. Genetic Fine-Mapping and Identification of Candidate Genes and Variants for Adiposity Traits in Outbred Rats. *Obesity*. 2018;26(1):213-222. doi: 10.1002/oby.22075.
- González-Domínguez Á, et al. Iron Metabolism in Obesity and Metabolic Syndrome. *Int J Mol Sci*. 2020;21(15):5529. doi: 10.3390/ijms21155529.
- Talbot F, et al. A rare human variant that disrupts GPR10 signalling causes weight gain in mice. *Nat Commun*. 2023;14:1450. doi: 10.1038/s41467-023-36966-3.