Genetic mapping of age-related hearing loss in CFW mice

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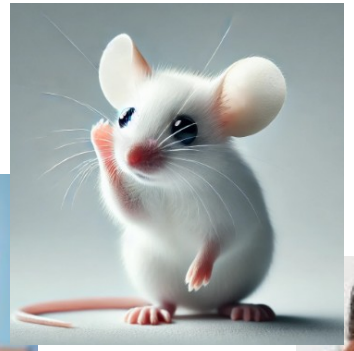
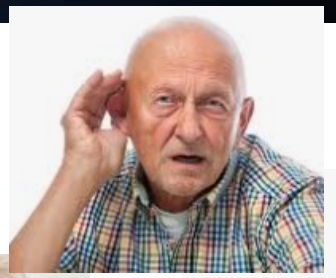
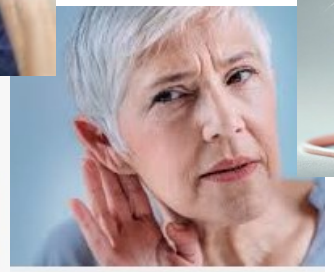
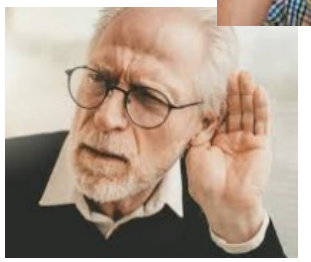
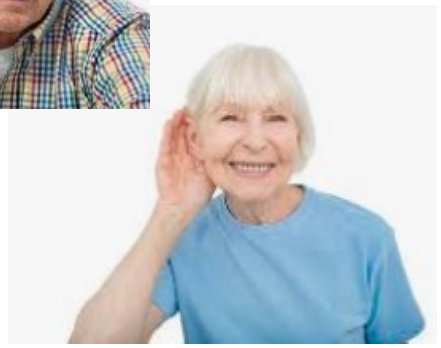
CTC 2024



Acknowledgements

Rick Friedman, MD, PhD - PI
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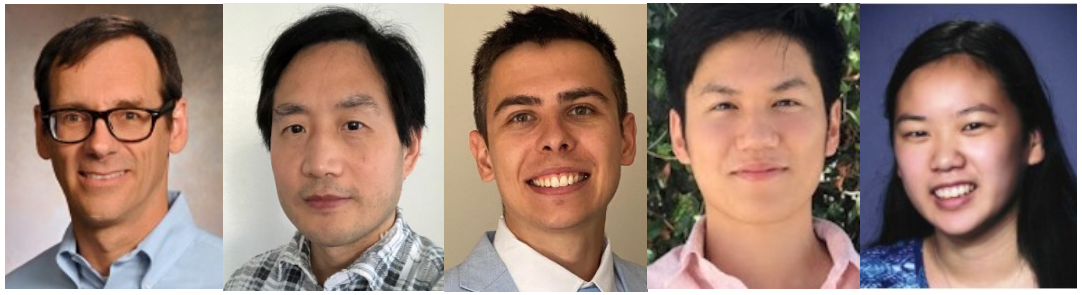
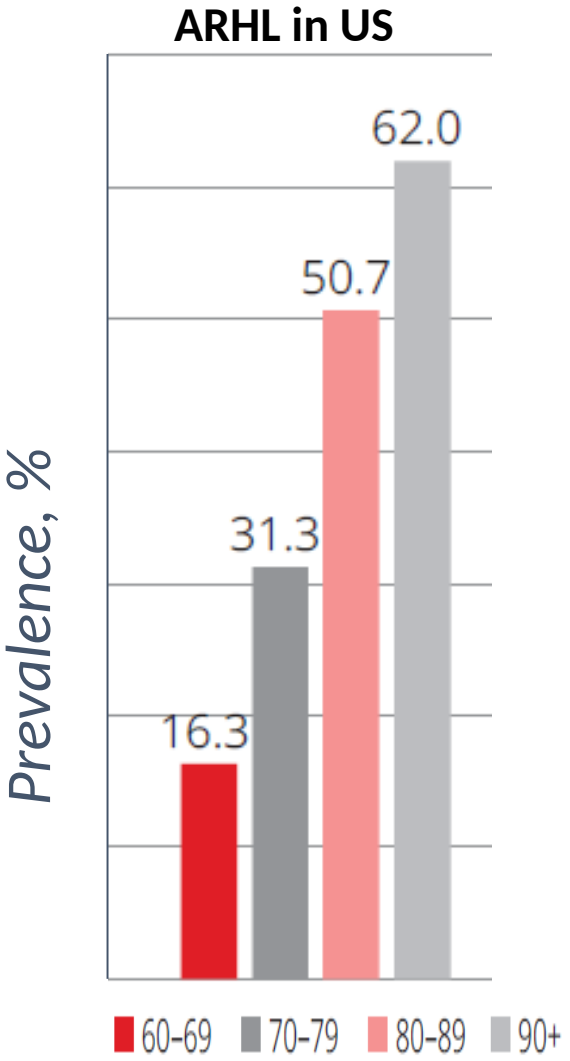


Abraham Palmer, PhD - PI
Khai-minh Ngyen - sequencing
Riyan Cheng, PhD - Genotypes
Thiago Sanches, PhD - GWAS
Mika Okamoto - summer intern

CFW for mapping of Age-Related Hearing Loss (ARHL)

- Problem: ARHL is an important health problem in US. The genetics of this condition is not clear.

- Tool: CFW outbred stock is a good population for genetic mapping
  - Many generations of outbreeding (>80 years!)
  - Rapid LD decay
  - Extensive genetic diversity within the population



ARHL Background

Humans:

- Twin studies: 25-75% heritability
- > 100 genes have been identified for monogenic deafness; however, a substantial fraction of patients have no identifiable mutation in any known deafness gene suggesting that there remain additional genes to discover
- The first human GWAS was done in 2009 (Friedman et al, 2009)
- UK biobank human GWAS identified multiple loci with genes related to inner ear pathologies, suggesting that there is an overlap between genetics of hearing loss in 2 species.

ARHL Background

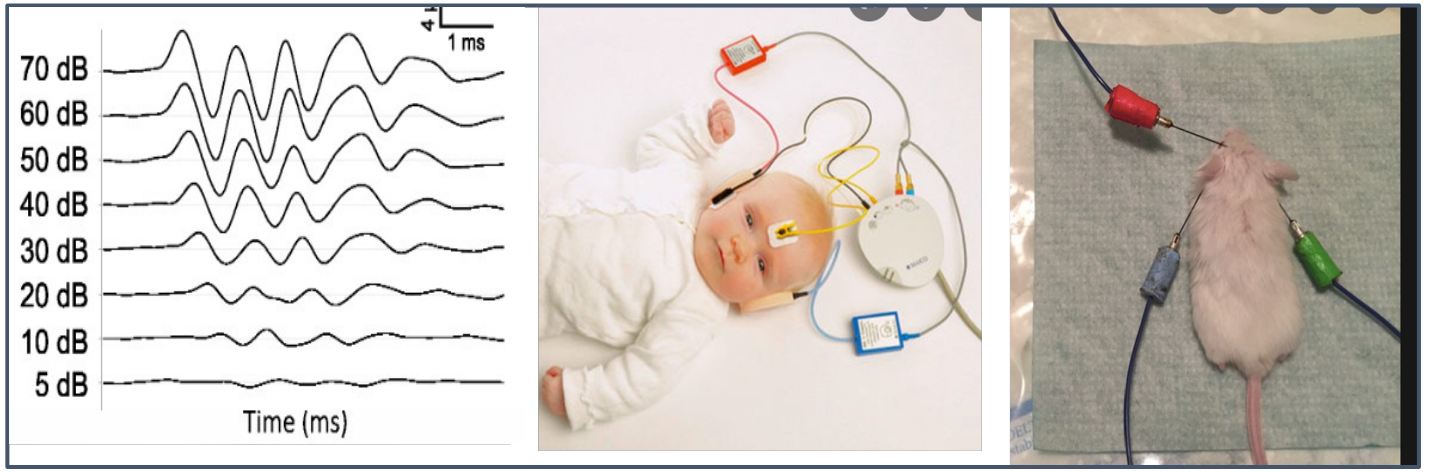
Mouse:

- This is the first GWAS in outbred mice
- Knockout Mouse Project/International Mouse Phenotyping Consortium (KOMP-IMPC) has identified 62 genes involved in early onset hearing loss by testing ABR thresholds in 14-week-old mice.
  <u>However:</u> young mice, null mutations, congenital rather age-related.
- Several ARHL loci were identified in BxD mice
- Several ARHL loci were identified in HMDP panel
- Several low-resolution hearing loss loci were identified by old fancy genetic methods.

Study design

Phenotype

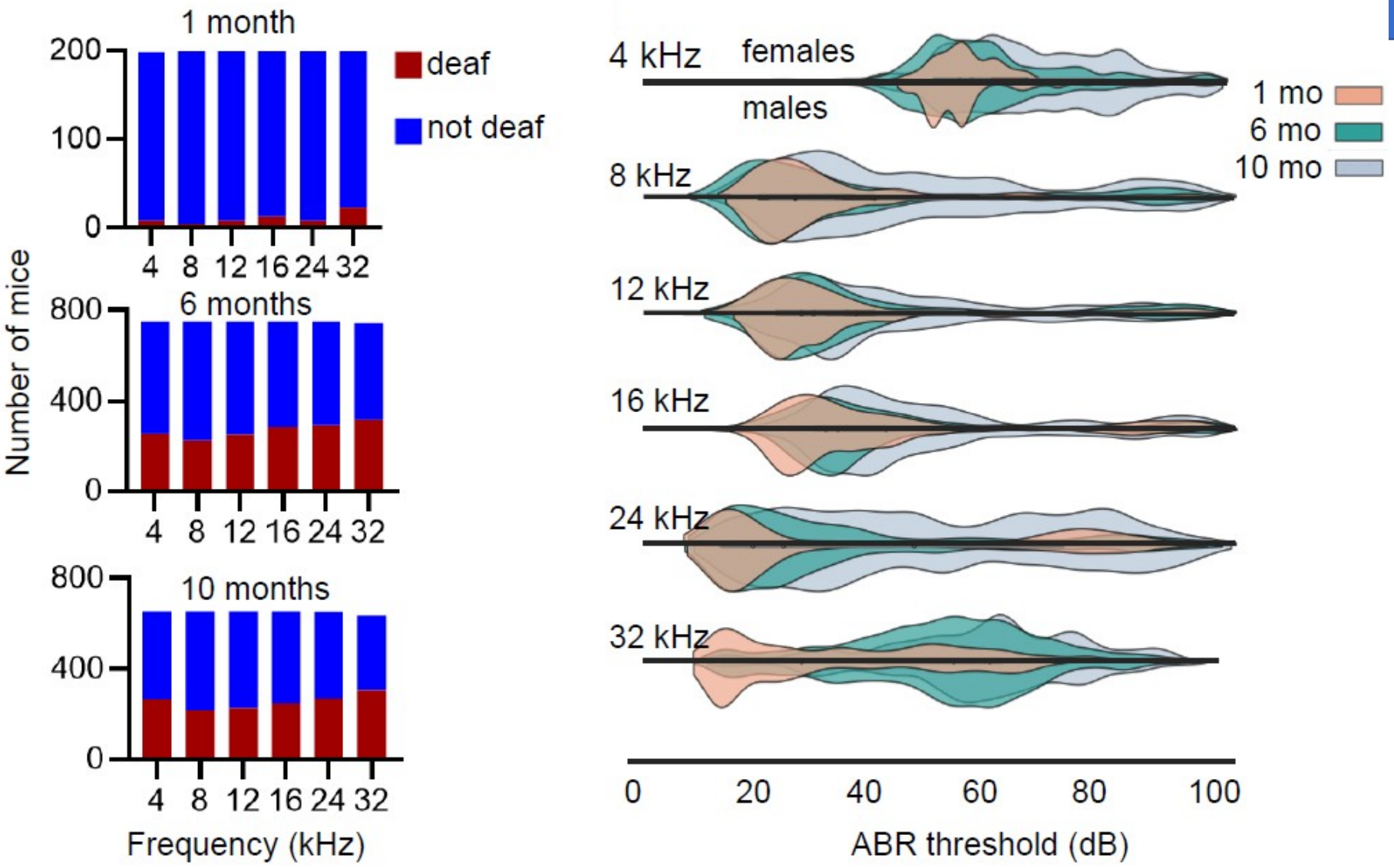
- Measure hearing as auditory brainstem response (ABR).
- Three time points:
  1, 6, 10 - month-old mice



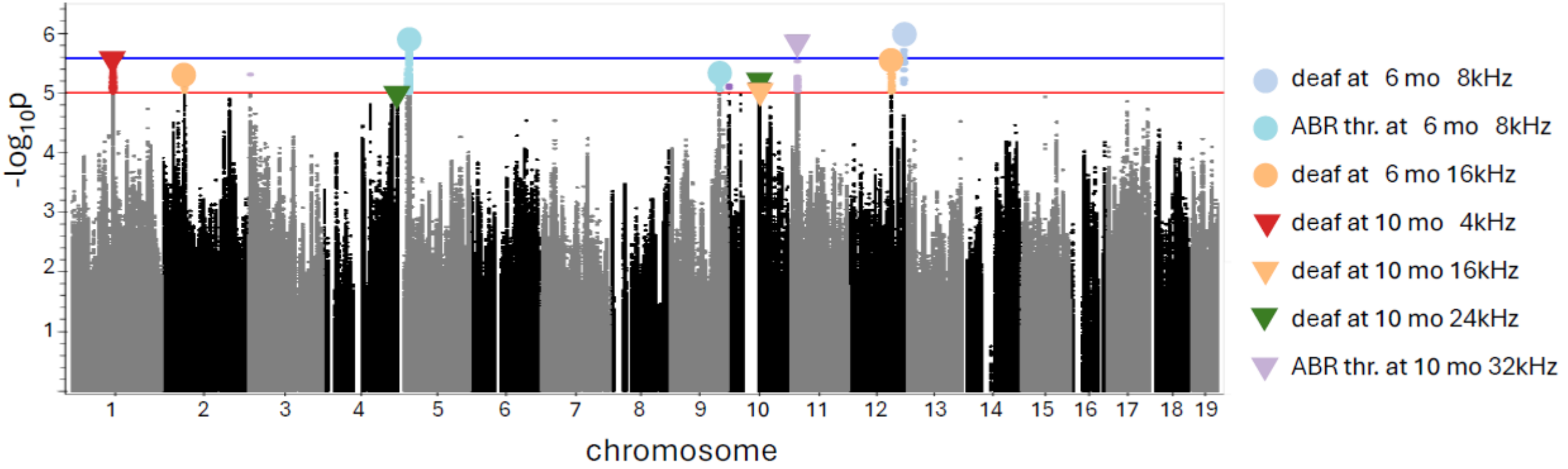
Genetics

- Use commercially available outbred CFW mice
- ~1000 M and F mice (target N = 2,000).
- Genotype using low-coverage WGS (~x0.3) followed by STITCH followed by BEAGLE imputation
- 2.15M autosomal SNPs, error rate 0.0053

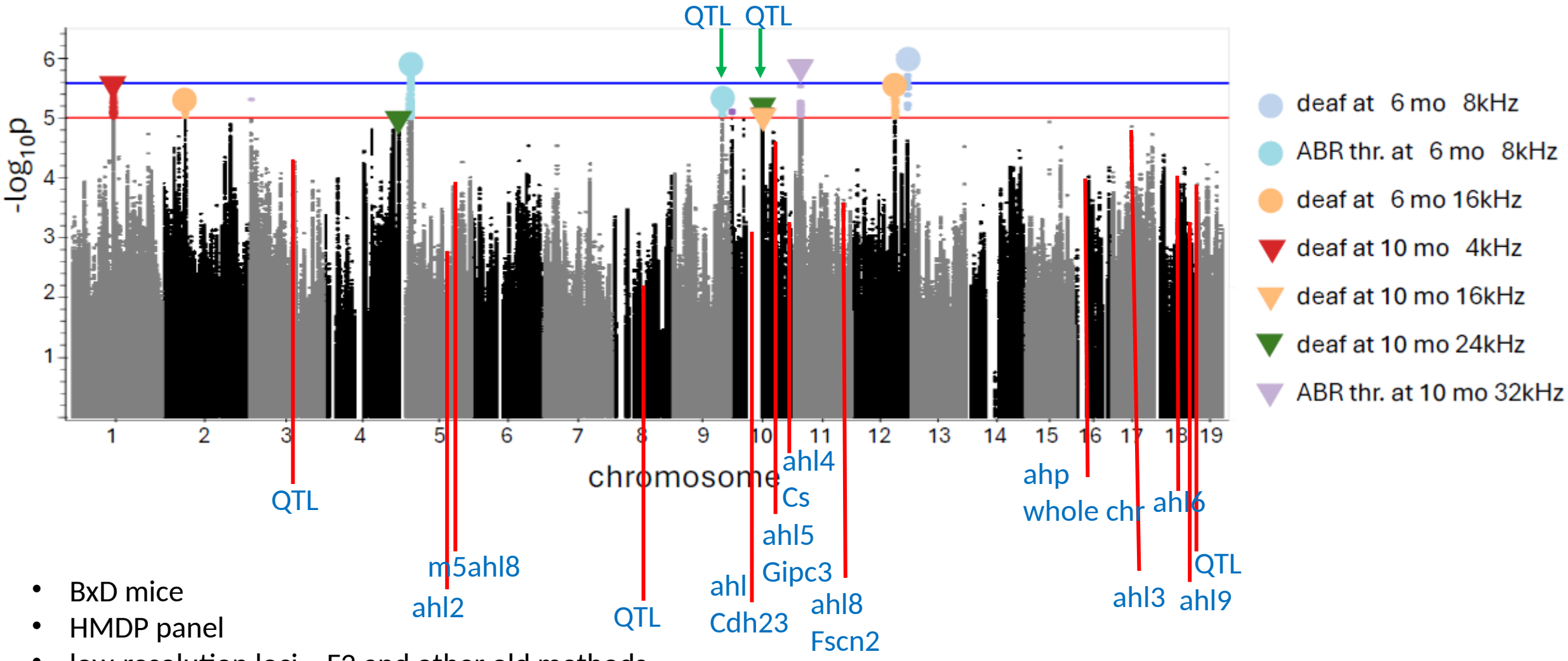
Phenotypes


1 month
200
100
0
deaf
not deaf
4 8 12 16 24 32
6 months
800
400
0
10 months
Number of mice
Frequency (kHz)
4 kHz
females
males
8 kHz
12 kHz
16 kHz
24 kHz
32 kHz
1 mo
6 mo
10 mo
0 20 40 60 80 100
ABR threshold (dB)

GWAS results



GWAS results:
replication of the previous studies



- BxD mice
- HMDP panel
- low-resolution loci – F2 and other old methods.

GWAS results:
replication of the previous studies

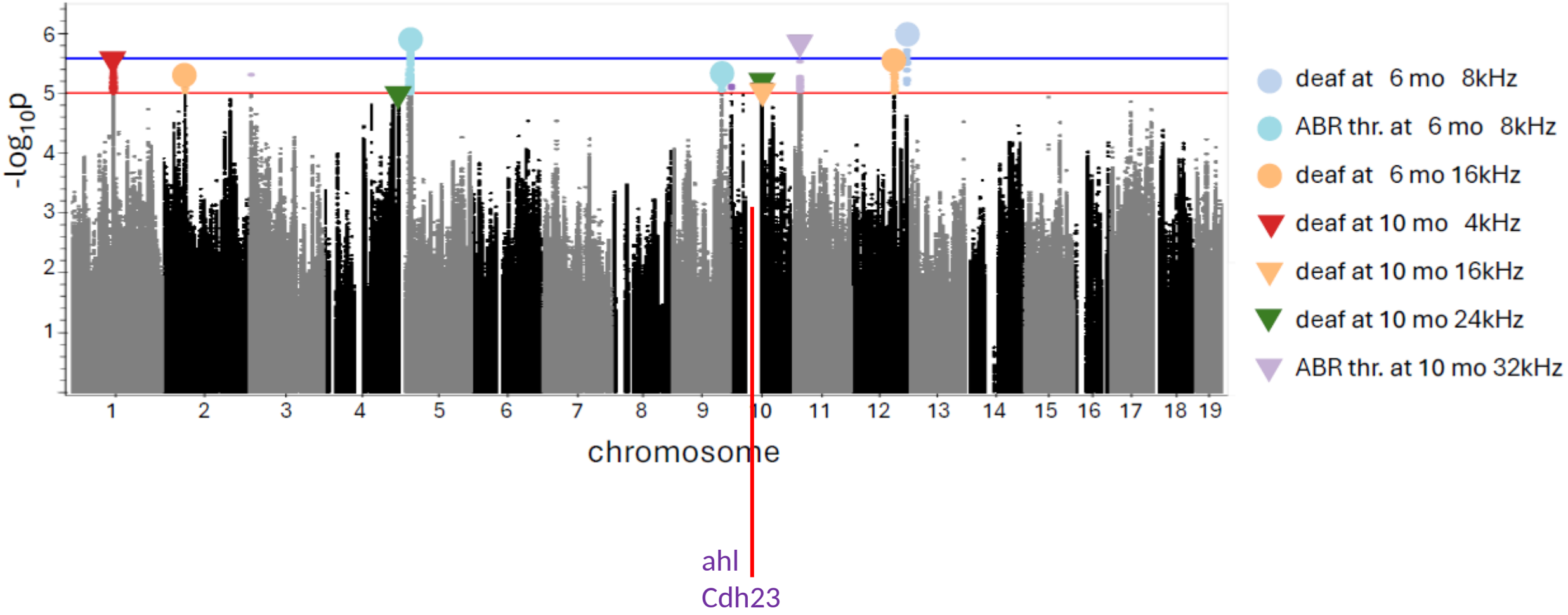
Why other findings were not replicated?

1) The alleles that are associated with deafness in other populations do not segregate in CFW stock

2) False positive in a previous study

3) False negative in our study

GWAS results



Chr 10 gap problem

Cdh23 variant is causing **early deafness**.
rs257098870 (Cdh23 chr10:60,530,947 G/A)
A is disrupting a splice site causing exon skipping

1) This mutation is present in many inbred mouse strains.
   Is it segregating in CFW?

2) It might explain early deafness in a proportion of CFW mice

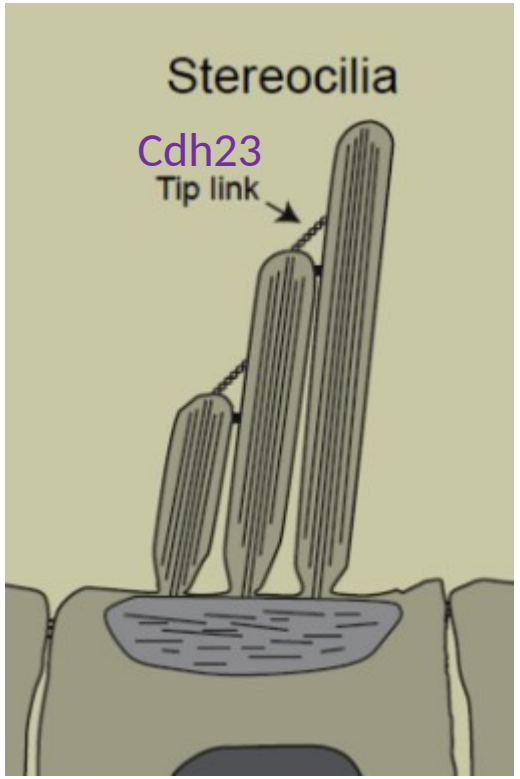


Image from:
Dionne, Gilman et al.
Neuron, 2018

Is rs257098870 segregating in CFW mice?

N = 86 mice have >=3 reads spanning chr10:60,530,947 and can be used to call this SNP using GATK.

MAF of A (deafness allele) is 0.63

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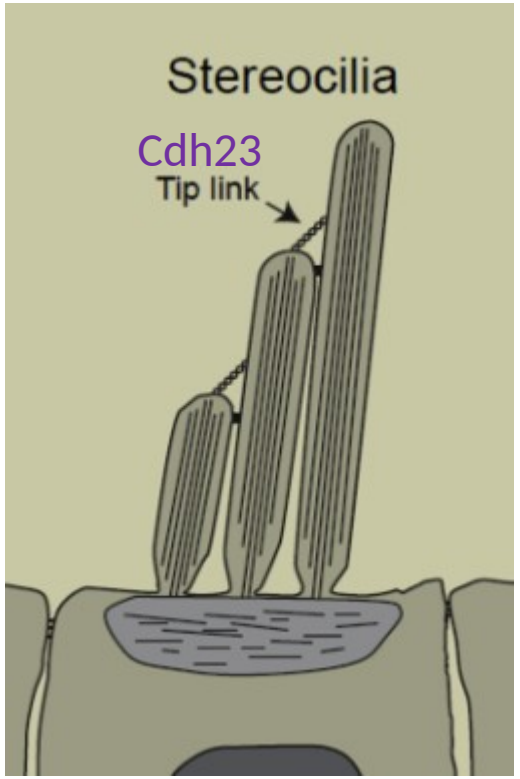
2) It might explain early deafness in a proportion of CFW mice



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2) Does rs257098870 explain early deafness in CFW mice?

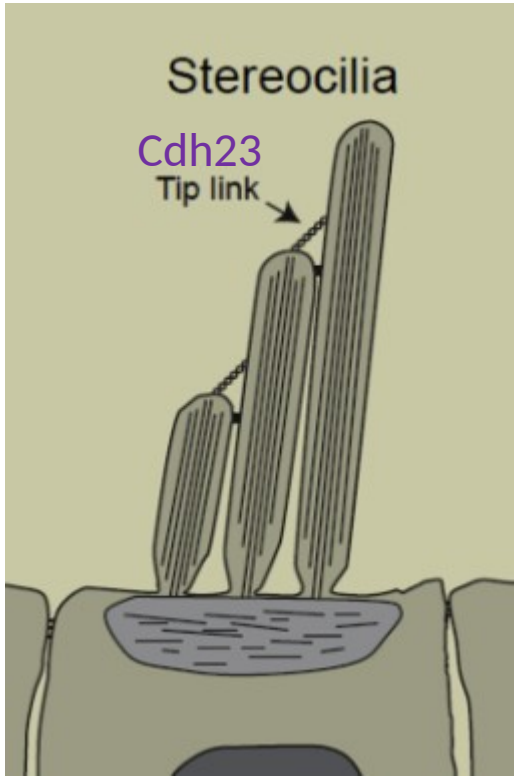
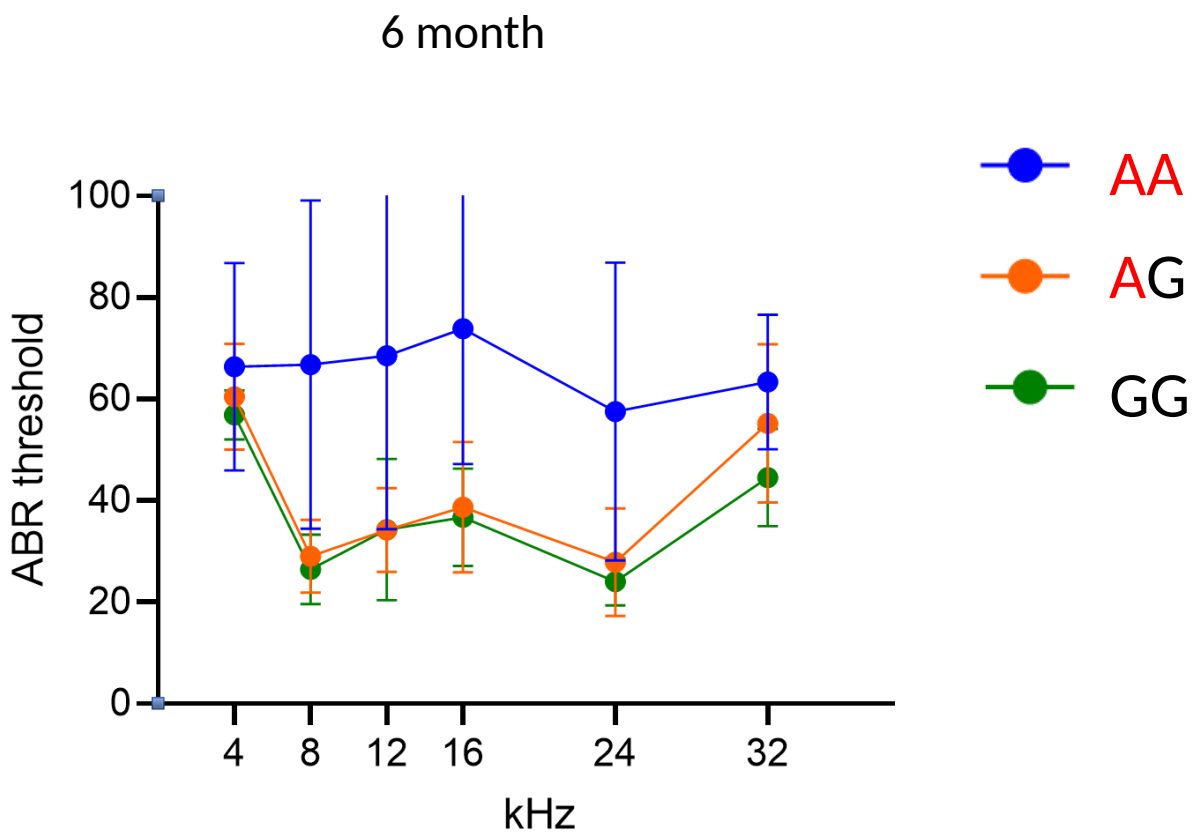


Image from:
Dionne, Gilman et al.
Neuron, 2018

Does rs257098870 explain hearing loss in CFW mice?



Chr 10 gap problem

Cdh23 variant is causing **early deafness**.
rs257098870 (Cdh23 chr10:60,530,947 G/A)
A is disrupting a splice site causing exon skipping

1) This mutation is present in many inbred mouse strains.
   Is it segregating in CFW? YES.

2) Does rs257098870 explain early deafness in CFW mice? YES, in part

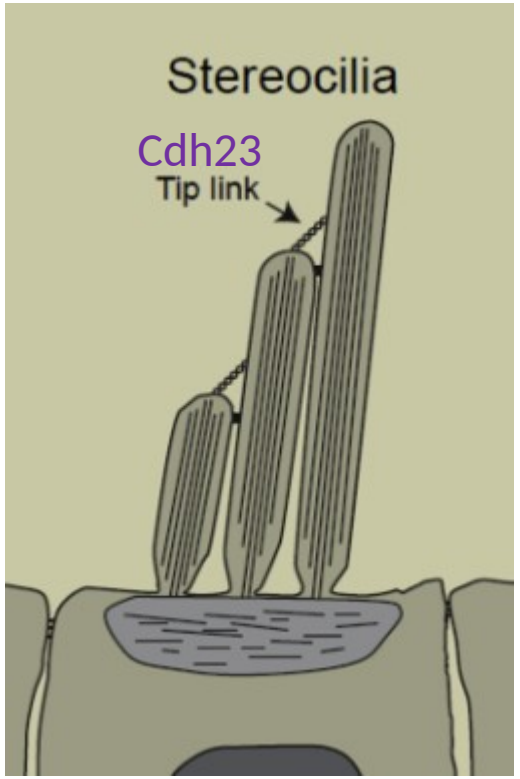
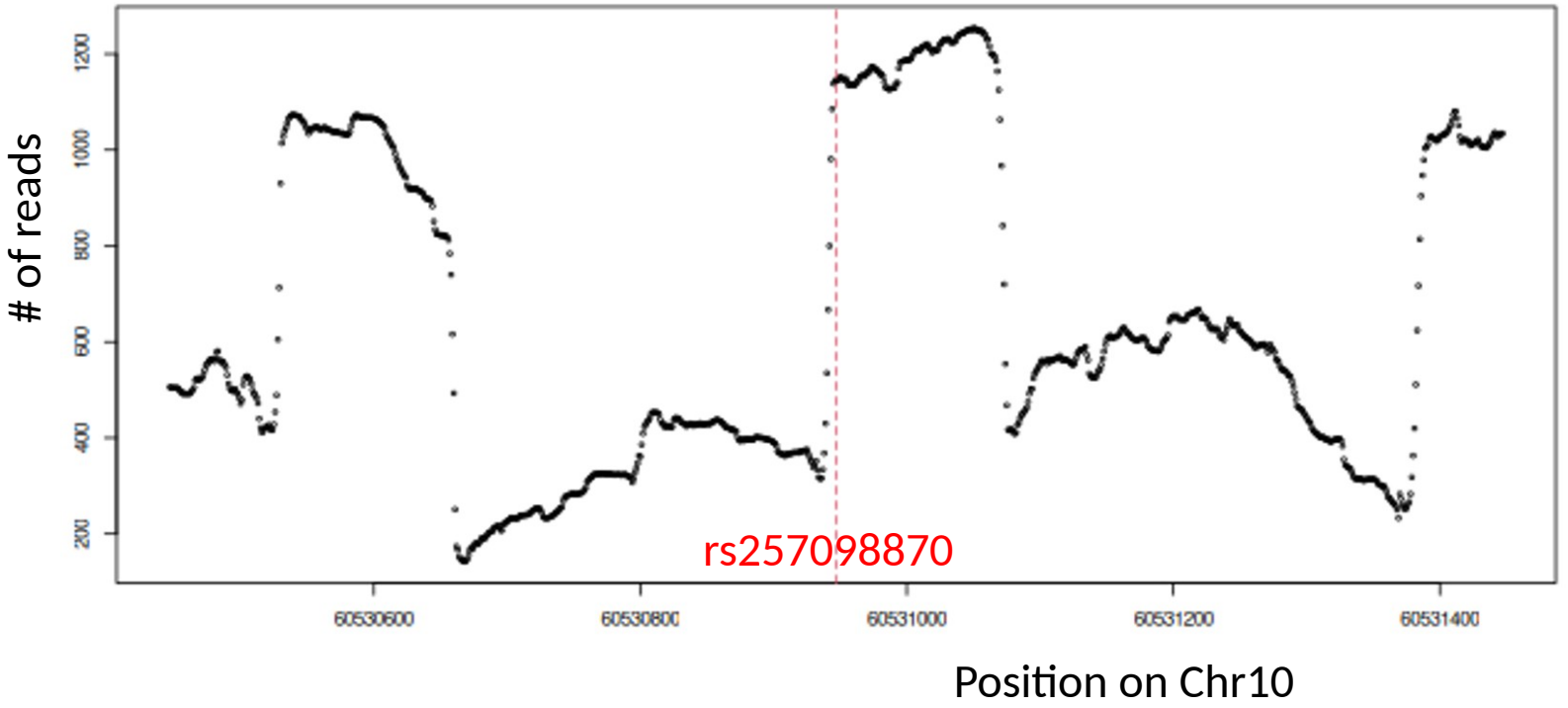


Image from:
Dionne, Gilman et al.
Neuron, 2018

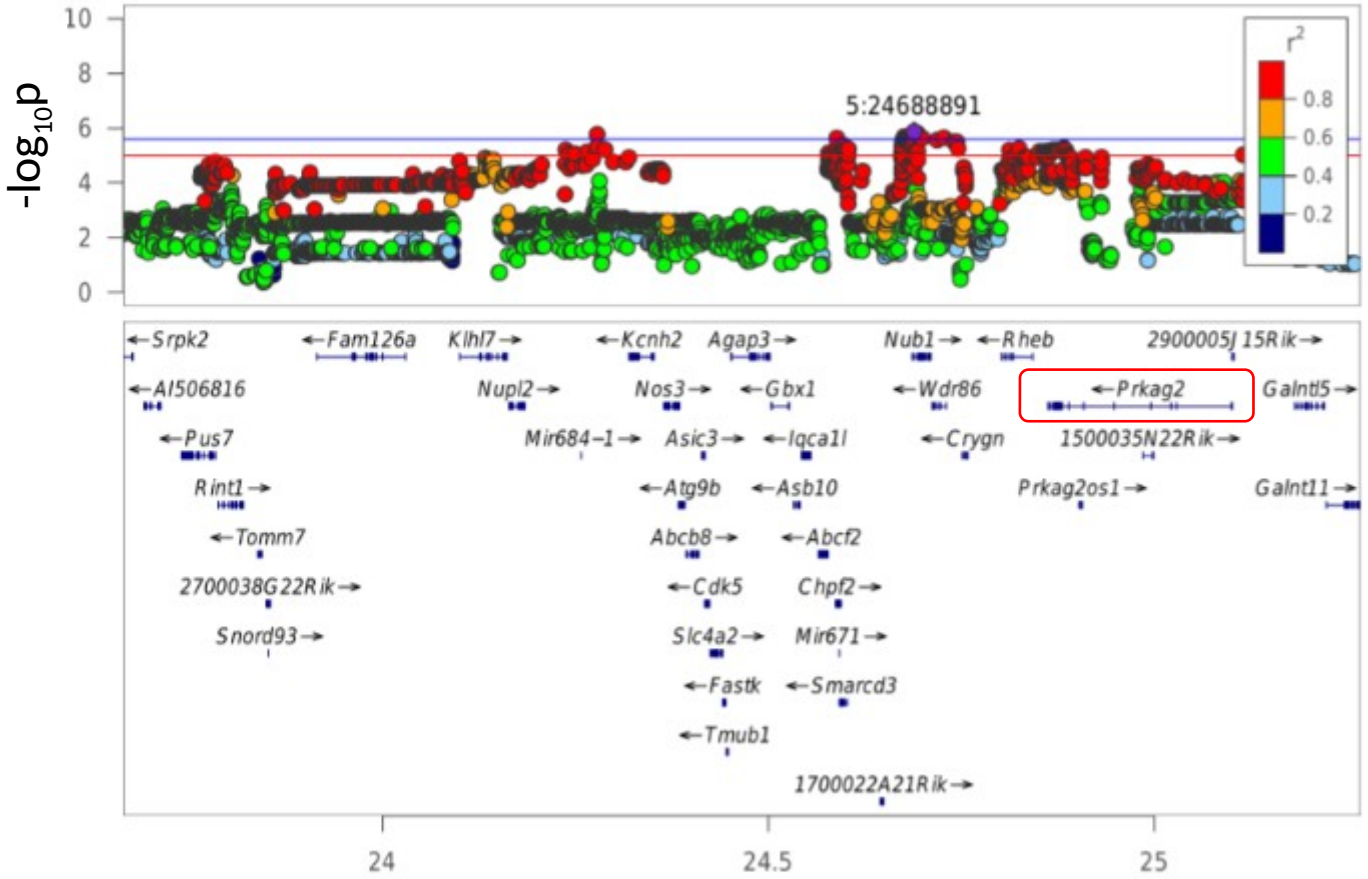
Why can’t we call rs257098870 with STITCH in mice sequenced with low coverage?

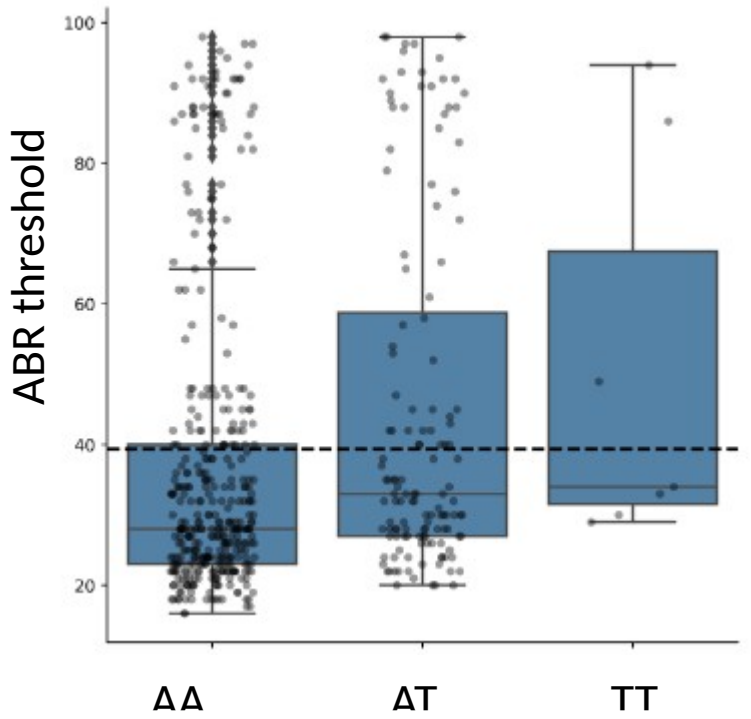
Reads from ~1000 lcWGS mice in 1 kb interval around rs257098870



rs 257098870 is near a structural variant in CFW

QTL on chromosome 5



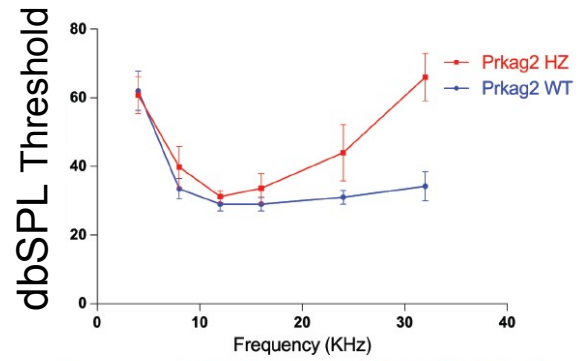


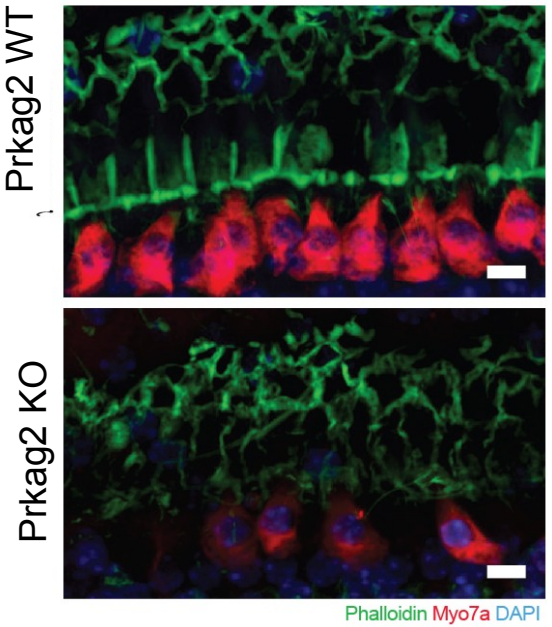
Prkag2 is a novel candidate gene

- *Prkag2* is a protein kinase subunit (5'-AMP-activated protein kinase subunit gamma-2). Known to be involved in hear development.

- *Prkag2* expression is detected in cochlea of 10 month old CFW mice (mostly in hair cells and spiral ganglion neurons)

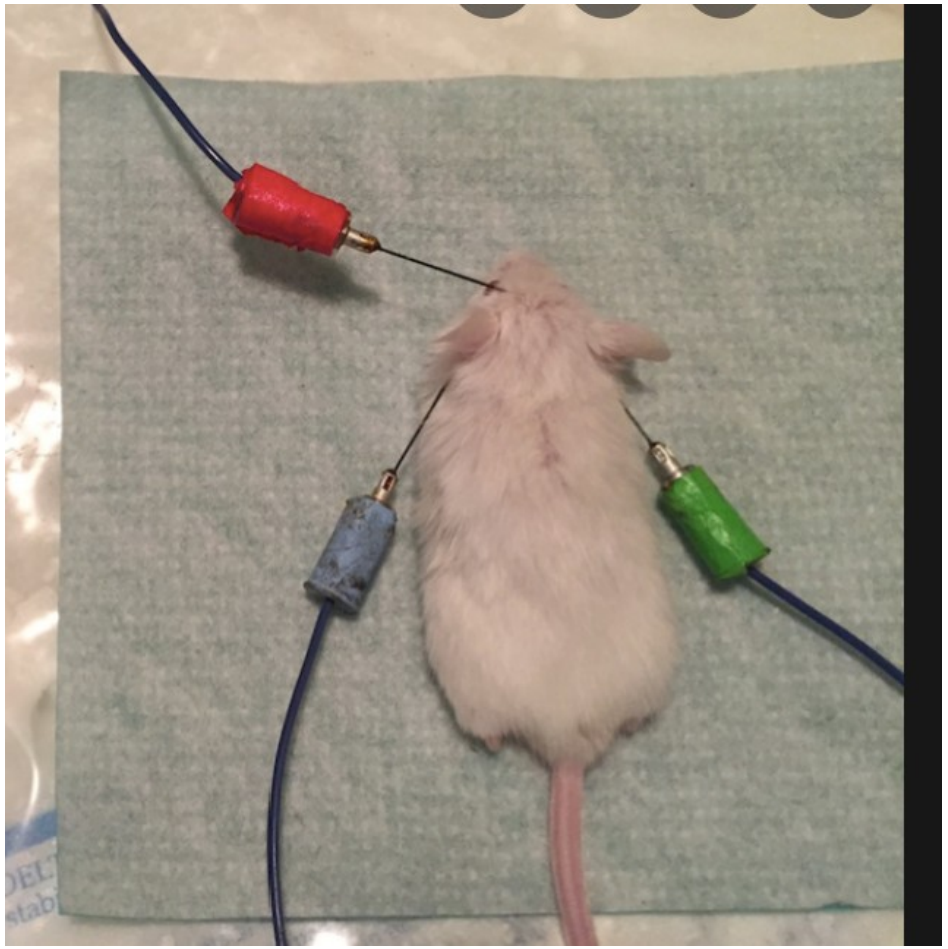
- *Prkag2* KO mice lose high frequency hearing
- In 2 years old mice WT littermates have better IHC preservation in comparison to KO mice

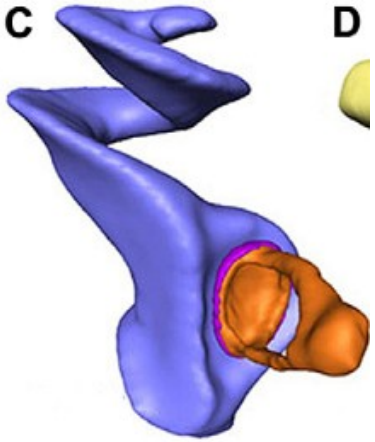


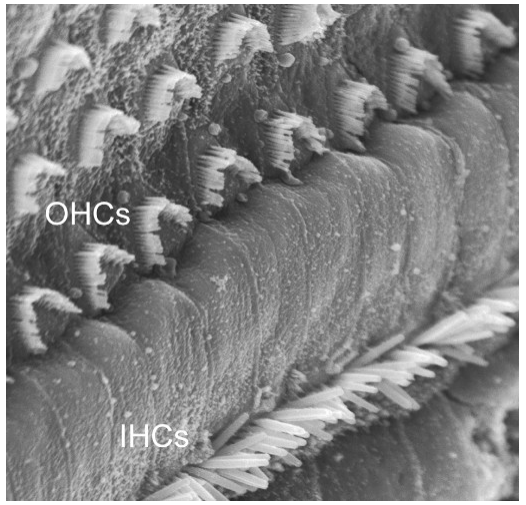


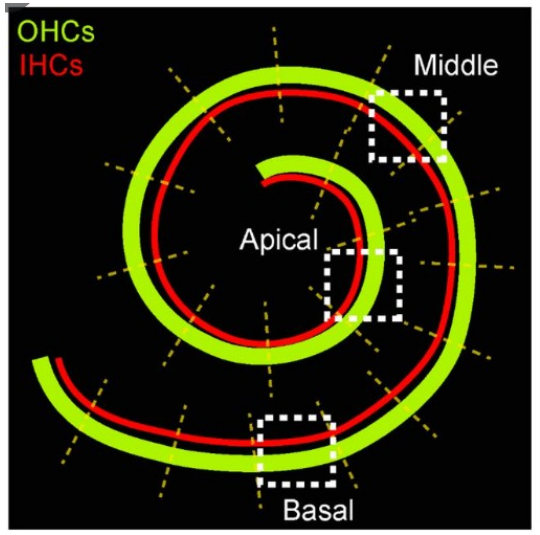
Conclusions and future directions

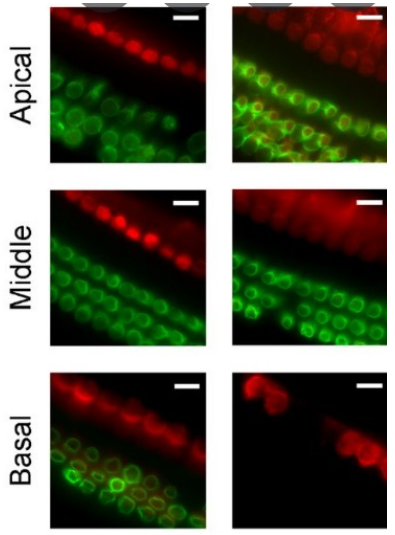
- ARHL is a highly polygenic disorder.
- We performed GWAS for ARHL in CFW mice; identified 10 loci containing plausible candidate genes.
- We need to figure out how to genotype rs257098870 to increase sensitivity of this GWAS
- We will collect data from the remaining mice, with a final sample size N~2,000, and perform genetic analysis on the full data set.




C
D


OHCs
IHCs

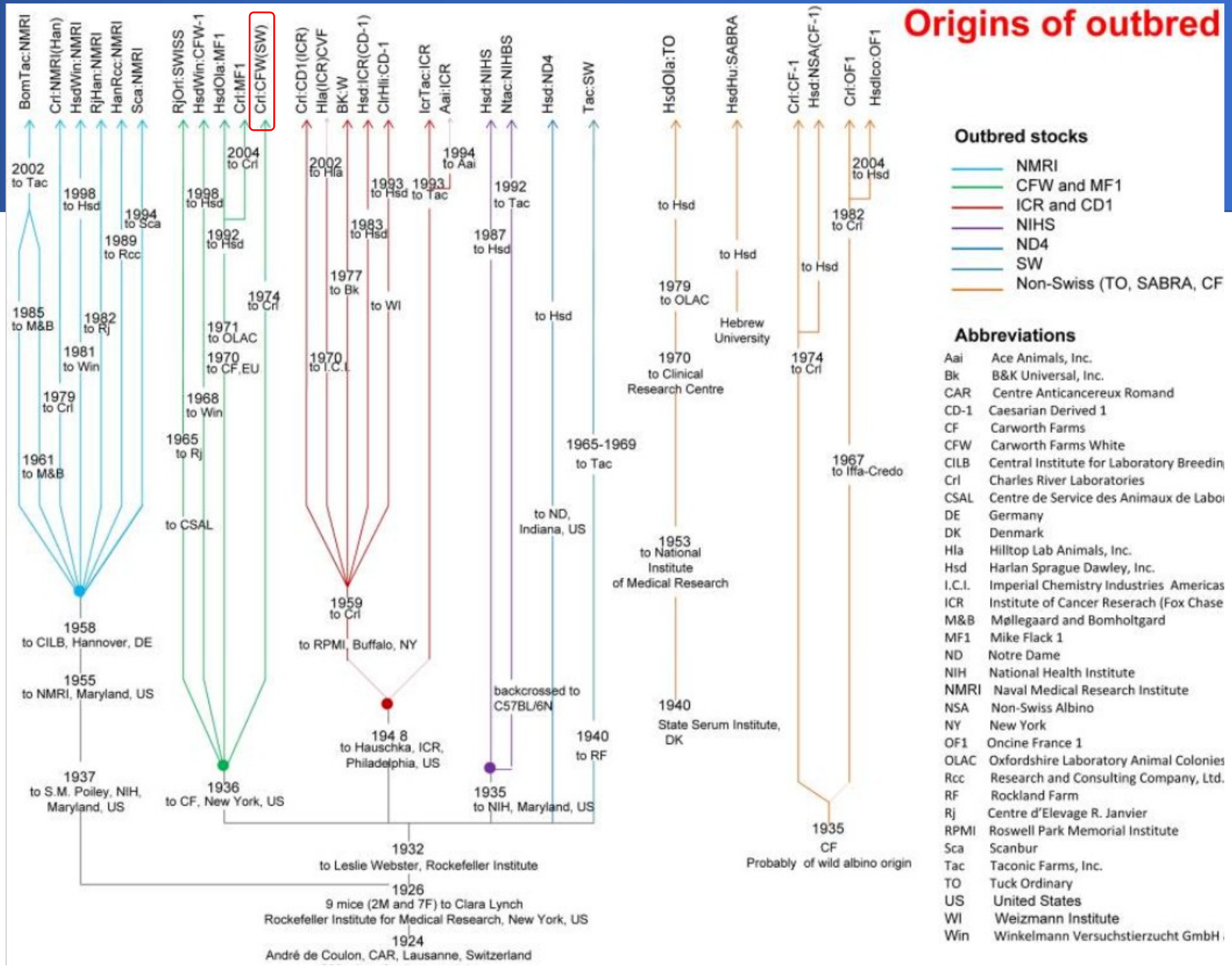

OHCs
IHCs
Middle
Apical
Basal


Apical
Middle
Basal

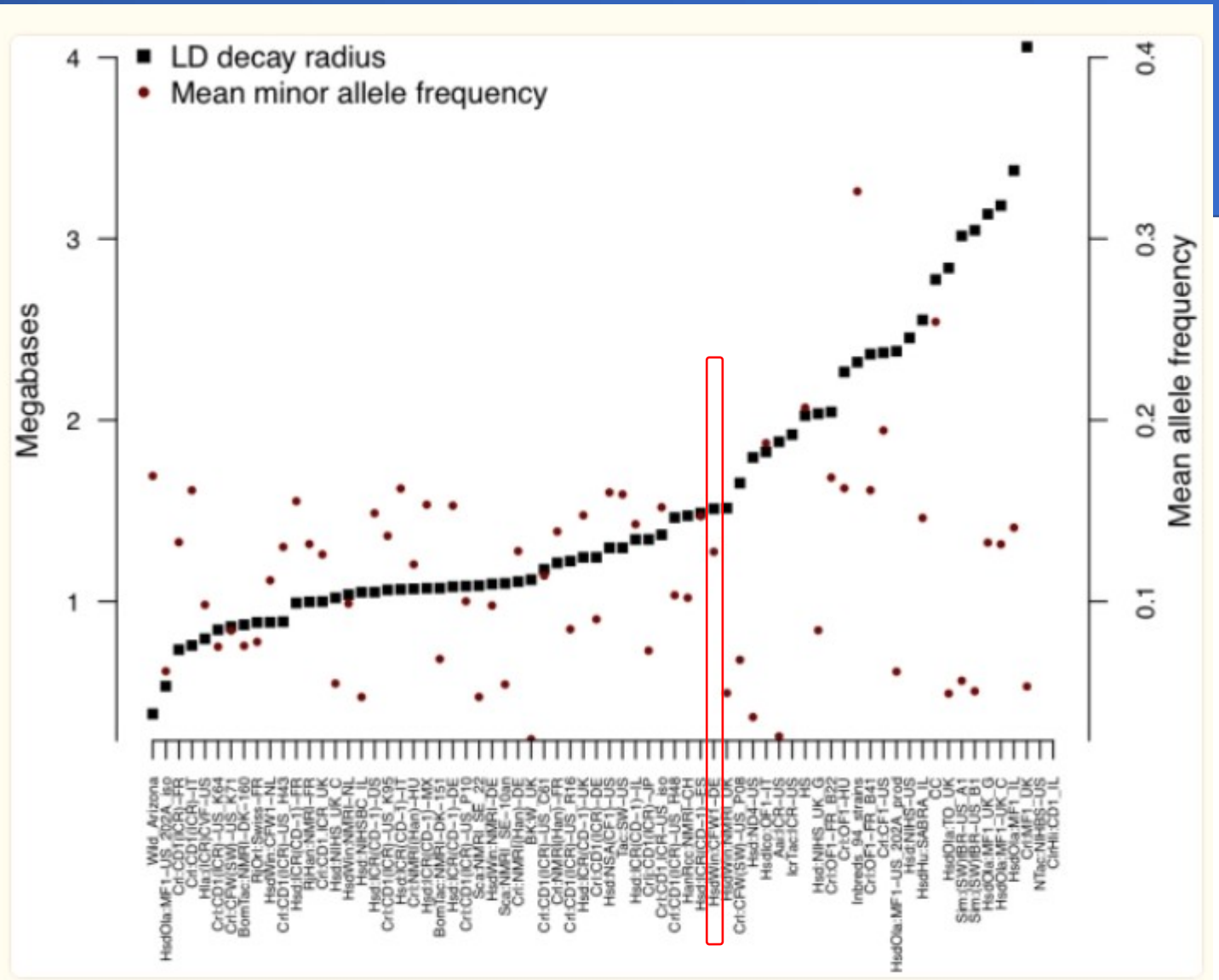
CFW history

Yalcin B … Mott R, Flint J. Commercially available outbred mice for genome-wide association studies. PLoS Genet. ***2010***

351 markers
two loci on chromosome 1 (131.6–134.5 Mb and 172.6–177.2 Mb),
one locus on chromosome 4 (136.2–139 Mb),
one locus on chromosome 17 (32.6–38.9 Mb)



CFW history: LD

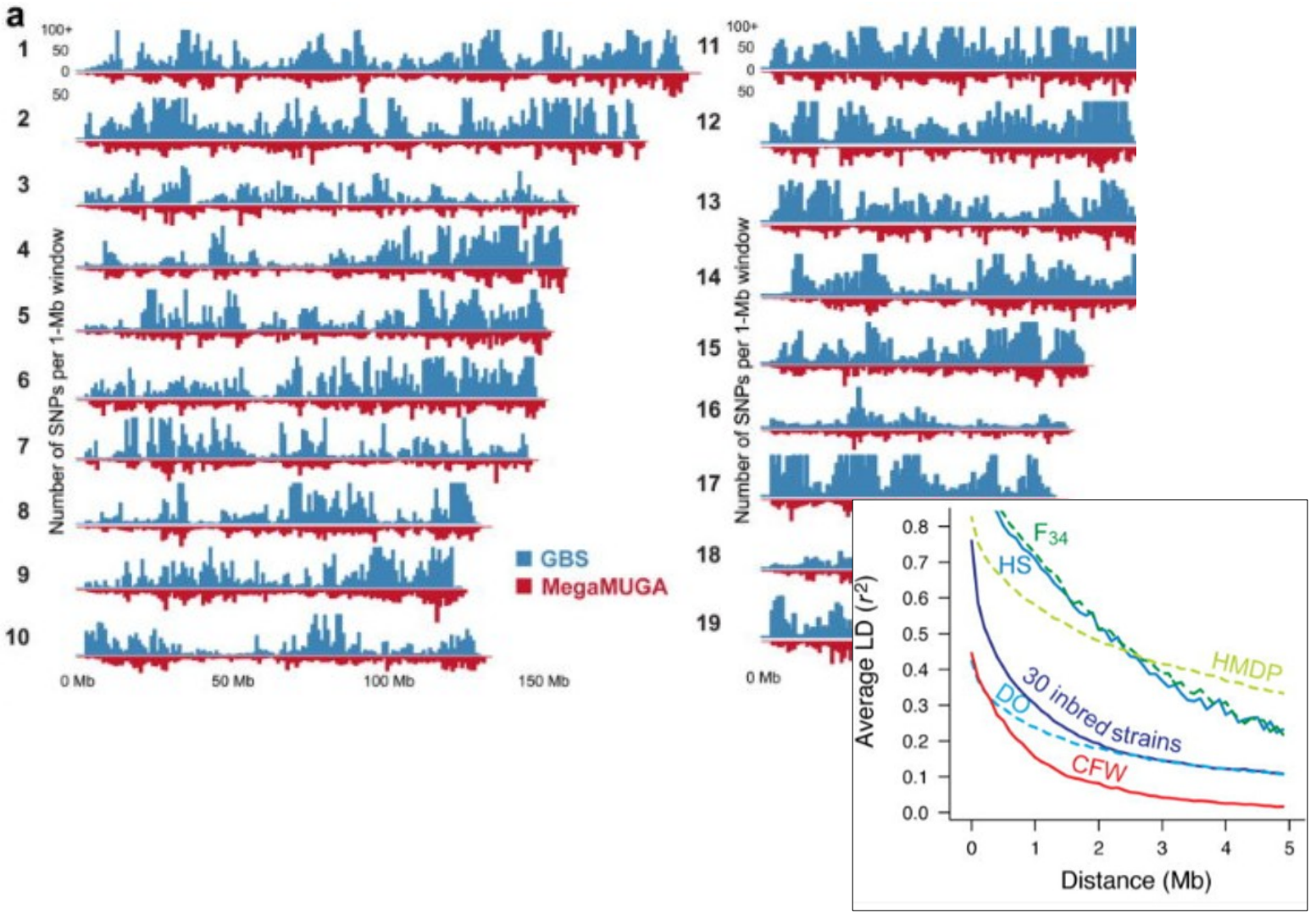


disequilibrium decay radius (black) and minor allele frequencies (red) in outbred mic

CFW history: polymorphisms

Parker CC et al., Genome-wide association study of behavioral, physiological and gene expression traits in outbred CFW mice. Nat Genet. ***2016***

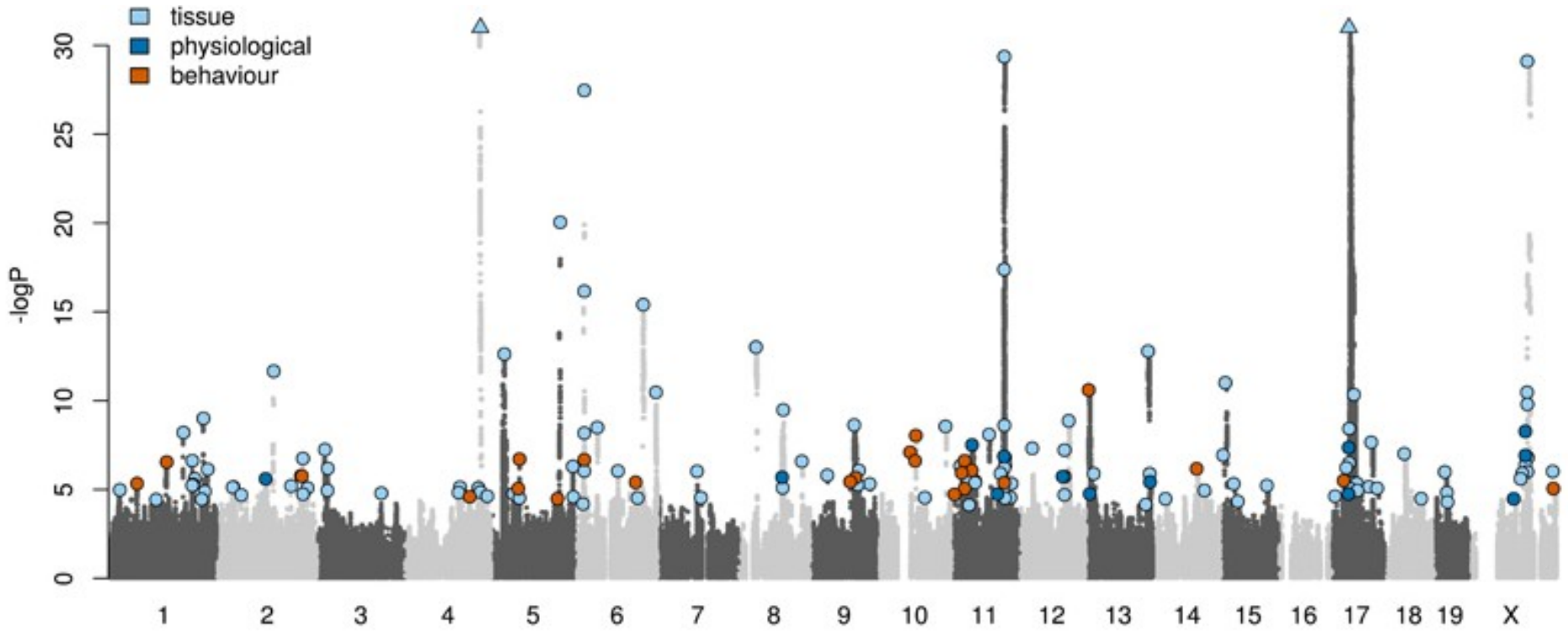
- GBS genotyping - 92,734 SNPs
- Mm38
- GATK used for discovering SNPs, filter for MAF>1% and missingness <0.95
- Reference generated from several WGS CFW mice+ known variants from other strains.
- IMPUTE2



CFW history: successful GWAS

Nicod J, Davies RW, …Mott R, Flint J. Genome-wide association of multiple complex traits in outbred mice by ultra-low-coverage sequencing. Nat Genet. ***2016***

0.15x coverage
STITCH (no reference)
5.7M SNPs

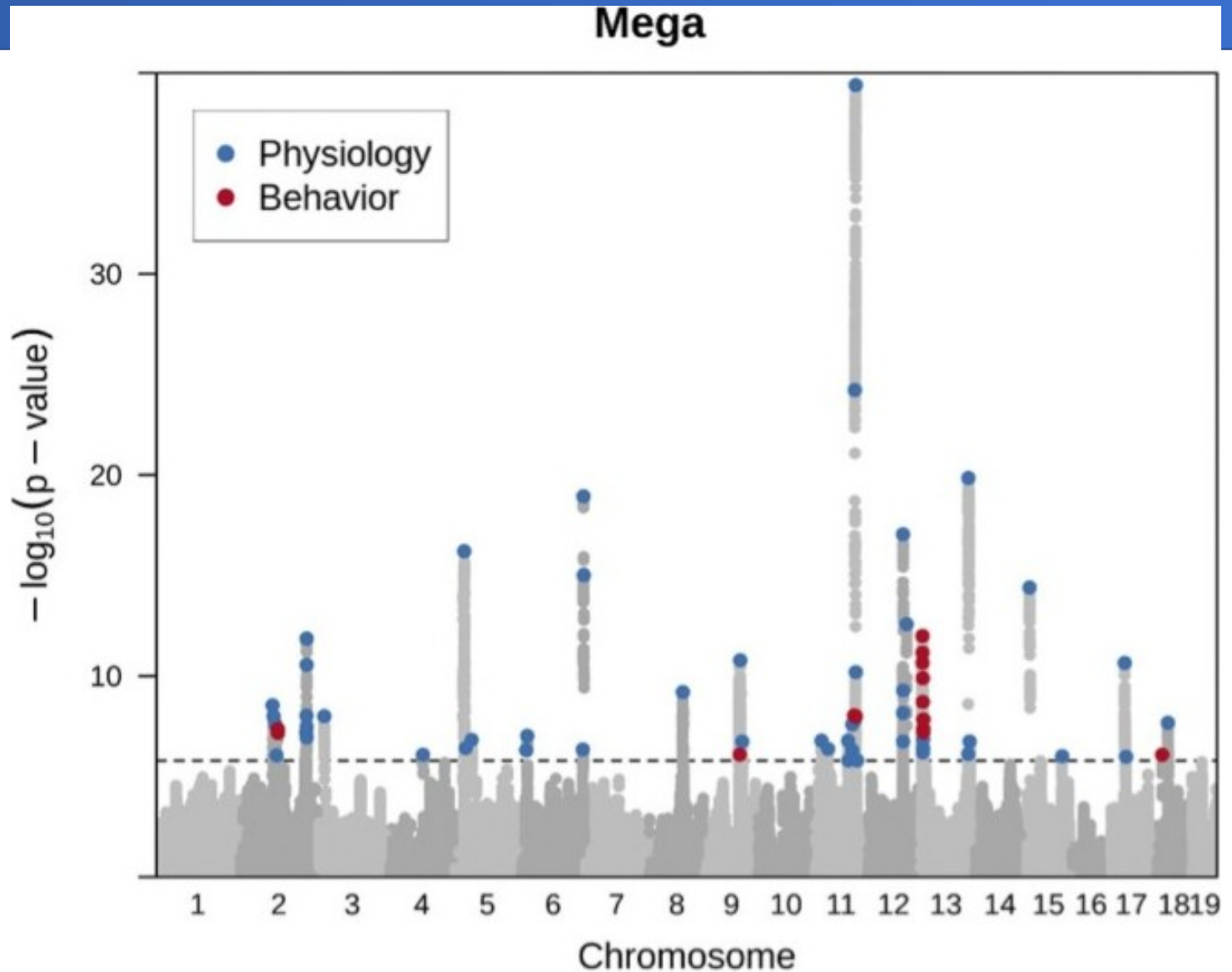


CFW history: successful GWAS

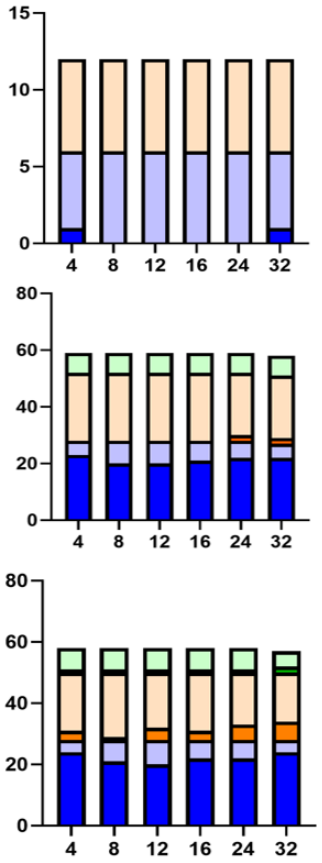
Zou J, …Palmer AA, Flint J. Analysis of independent cohorts of outbred CFW mice reveals novel loci for behavioral and physiological traits and identifies factors determining reproducibility. G3 (Bethesda). ***2022***

Common set of 3,152,108 SNPs for mapping with MAF > 0.001.
Concordance with MEGA Muga: 97.14% (Ox), 98.71% (UC).

After filtering the imputed genotypes for pairwise r^2 (>0.999), **97,452 SNPs** were retained for mapping



Deafness



AA
deaf
not deaf

AG
deaf
not deaf

GG
deaf
not deaf