NOV. 6, 2019 • BCERP ANNUAL MEETING

Using the Diversity Outbred mice to identify gene by environment interactions

Alison Harrill, PhD

Uncovering environmental cancer causes @ National Toxicology Program

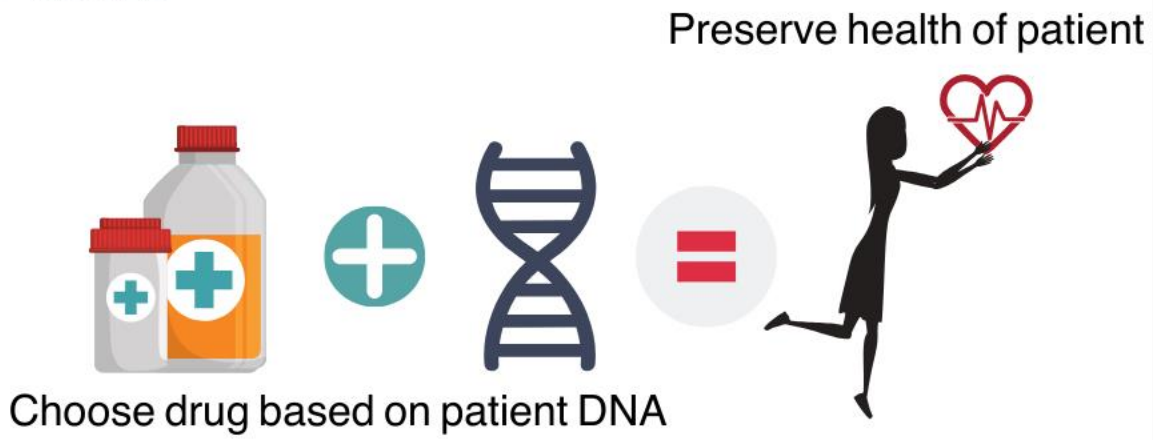
Identify environmental causes of cancer subtypes (ex. early onset colorectal cancer, breast cancer)

Protect public health by informing regulators of potential chemical threats and their potencies

Preserve patient quality of life via Precision Medicine

Toxicology - Study how drugs cause injury to tumor and healthy tissue

Pharmacogenetics - Determine which genetic sequences affect drug response in individual patients



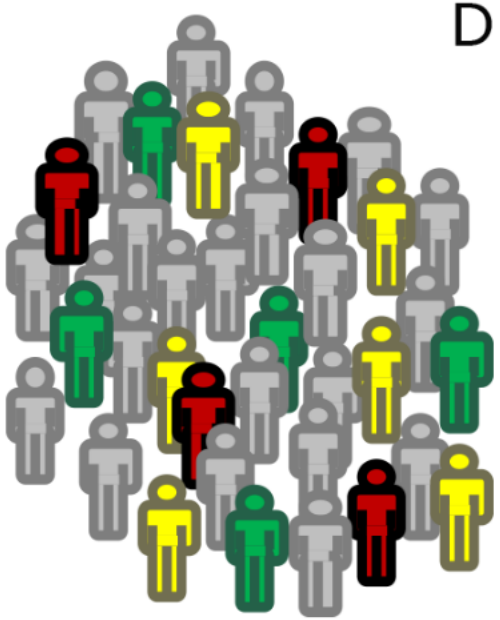
PRECISION TOXICOLOGY - MINIMIZE COLLATERAL ADVERSE EVENTS

Pharmacogenomics (PGx)

Seeks to understand how a patient's gene sequence affect how she responds to a medication (or chemical)

Drug causes adverse event, but is efficacious

Drug is not toxic and not efficacious



Same diagnosis,
Same prescription

Drug causes adverse event and not efficacious

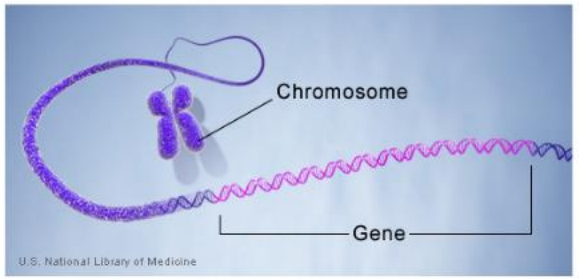
Drug not toxic, but is efficacious

Harrill Book Chapter 2016

Genetic Terms & Definitions

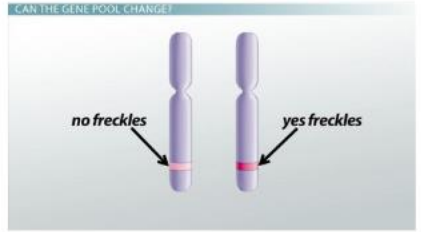
GENE

A direct sequence of nucleotides forming part of a chromosome



ALLELE / GENOTYPE

One of 2 or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome



SINGLE NUCLEOTIDE POLYMORPHISM

An allele that encompasses a single nucleotide in the sequence

Person 1 ATGCTTGGCGTA
Person 2 ATGCATGGCGTA

MAJOR ALLELE

The common allele / variation / nucleotide

MINOR ALLELE

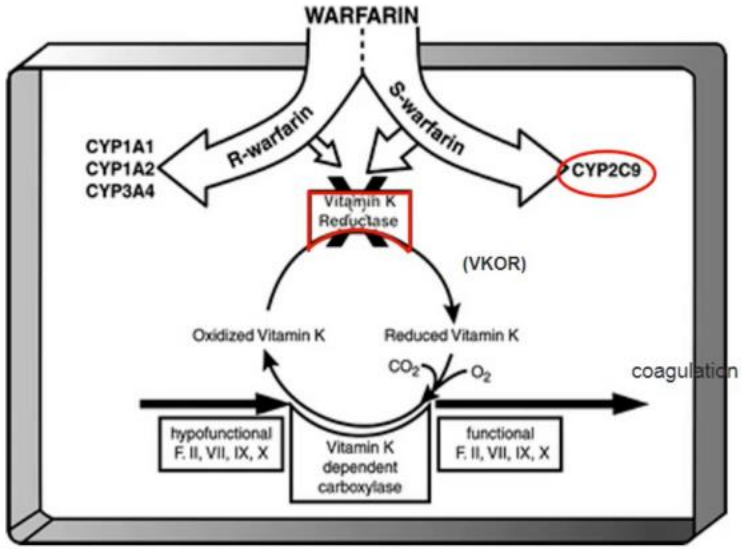
The less common allele / variation / nucleotide

95% People ATGCTTGGCGTA
5% People ATGCATGGCGTA

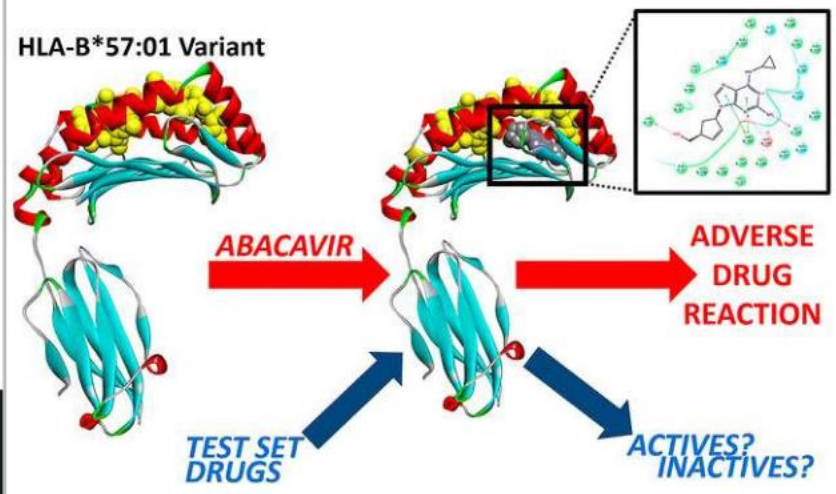
PHENOTYPE

A set of observable characteristics arising from the individual's genotype and its interactions with the external environment

Genetic Sequence Variability Can Affect Response to Drugs in Several Ways



Adapted by permissions from Macmillan Publishers Ltd: [The Pharmacogenomics Journal] (V.4: Issue 4, 224-225), (pg.225), (2004)



Phys.org NC State 2017

PHARMACOKINETICS

plasma clearance, distribution of drug to target organs/cells, metabolite production

Example: Warfarin dose setting is guided by PGx test for CYP2C9 and VKORC1

PHARMACODYNAMICS

Genes down or upstream of metabolism play a role in modulating drug effect

Example: Direct binding of abacavir to HLA-B*5701 causes allergic skin rash

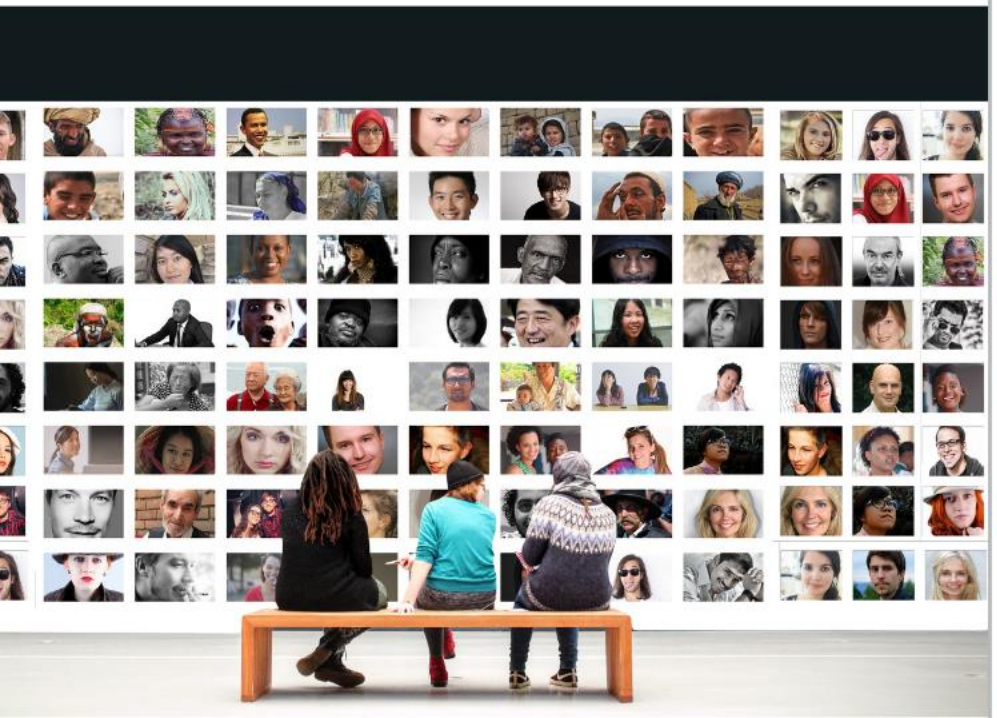
GUIDE TREATMENT

Your cancer drug has been found to cause an adverse reaction in a subset of patients. A pharmacogenomic test can allow the drug to reach patients who would benefit, while minimizing risks for those who would be harmed

PREVENT ENVIRONMENTAL CAUSES OF CANCER

You want to determine the mode of action of sensitivity to the drug/chemical. Alternatively, you want to know if there are shifts in dose response across the population that can affect safe human exposure thresholds of environmental chemicals

Genetic differences between patients are important to understand



A Problem

MOST TOXICOLOGY STUDIES PERFORMED BEFORE DRUGS MOVE TO CLINICAL TRIALS ARE DONE IN GENETICALLY LIMITED ANIMALS

We typically do not test drugs on genetically diverse populations of animals -> that means we will miss individual differences in response due to different alleles (versions of gene sequences)

Utility of Animal Models

FOR TOXICOLOGY

CONTROLLED EXPERIMENTS

Can set variables: timing and dose of drug/chemical administration, diet, environment/stress, known genetics

EXPERIMENTS CAN BE REPLICATED

We standardize reagents (ex 99.9% pure compound) - animals can also be thought of as a reagent and should be well-defined

ETHICS

We don't want to dose humans with a chemical of unknown toxicity

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COMPARATIVE GENOMICS

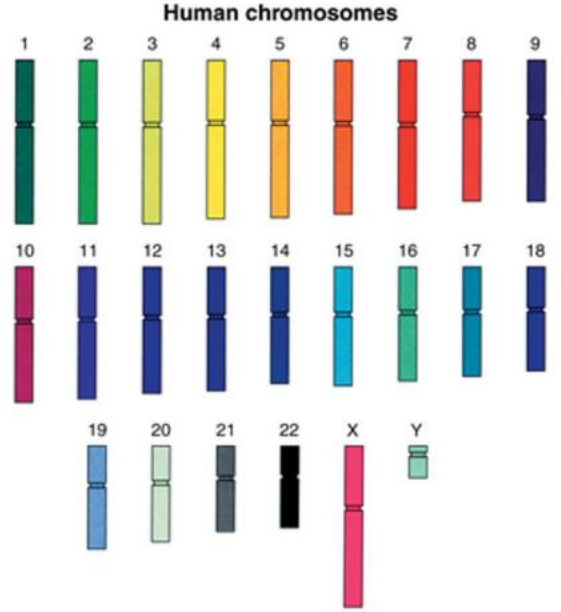
Utility of Animal Models (GxE)

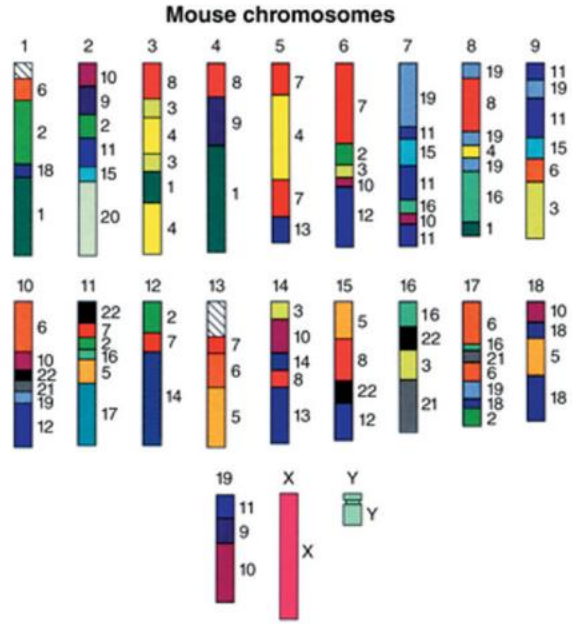
FOR PHARMACOGENOMICS

Humans and mice share 92% genetic sequence homology

GENETIC SYNTENY

Gene sequence order is rearranged between mouse and human





Like humans, mice have migrated across globe with gene flow across continents

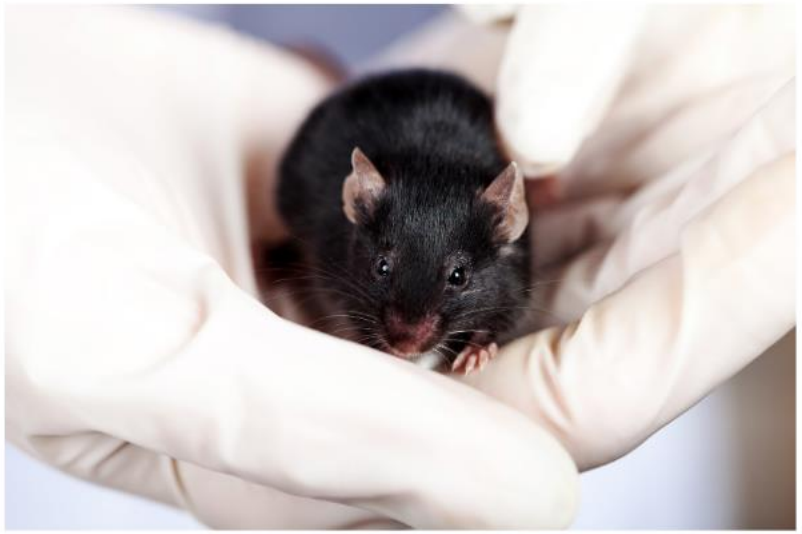
Subspecies of mice/rats have a varied geographic origin

Inbred Strains Characteristics

- GENETICALLY IDENTICAL
- HOMOZYGOUS AT MOST LOCI

  No difference in the allele that comes from mom vs the one that comes from dad
- LOW PHENOTYPIC VARIABILITY

  Assuming all conditions and environment are the same/ well-controlled
- LOW N NEEDED TO POWER STATISTICS
- EASY TO MAINTAIN GENETIC FIDELITY



Inbreeding can lead to genetic disease and poor health status, which is one reason these are favored as disease models

Outbred Stock Characteristics

- GENETICALLY UNIQUE INDIVIDUALS
- HETEROZYGOUS AT MANY LOCI

  Better mimic human populations because this is common for us too

- HIGH PHENOTYPIC VARIABILITY

  Different shapes and sizes, just like people

- HIGHER N NEEDED TO POWER ANALYSIS

  Tradeoff for having greater phenotypic diversity

- MUST DILIGENTLY MAINTAIN GENETIC FIDELITY

Outbred populations mimic human diversity better than single inbred strains

Models with utility for assessing gene by environment interactions to assess AEs associated with chemotherapy

USE A PANEL OF COMMON INBRED STRAINS

Presentations are tools that can be used as lectures.

[redacted]

USE RECOMBINANT INBRED STRAIN PANEL

Presentations are tools that can be used as lectures.

[redacted]

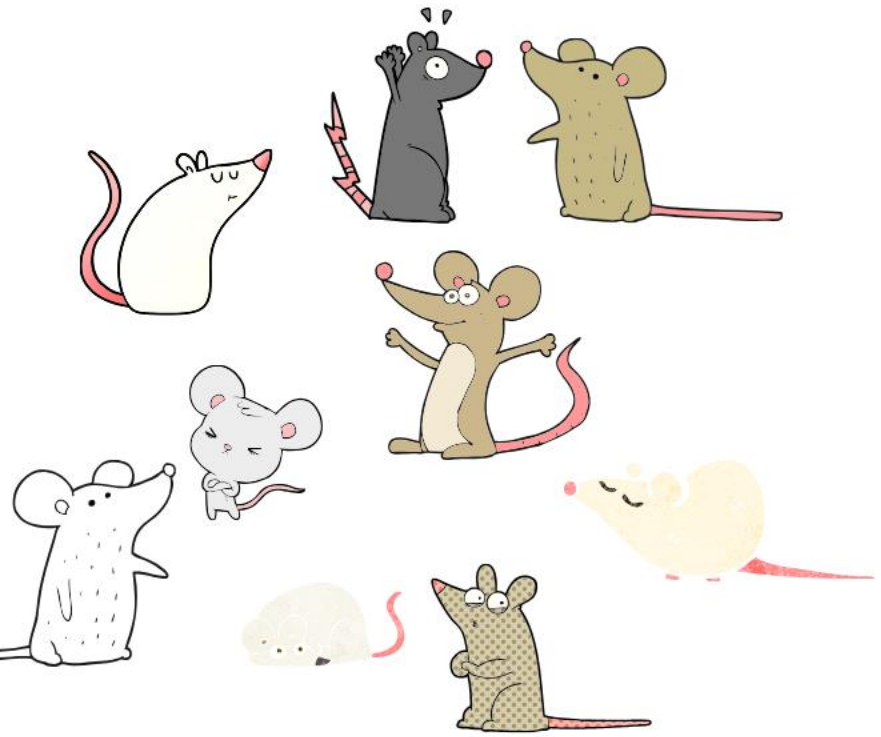
USE A HIGHLY DIVERSE OUTBRED STOCK

Presentations are tools that can be used as lectures.

[redacted]

GENETICALLY DIVERSE OUTBRED MOUSE STOCK

Different mice > different genetics



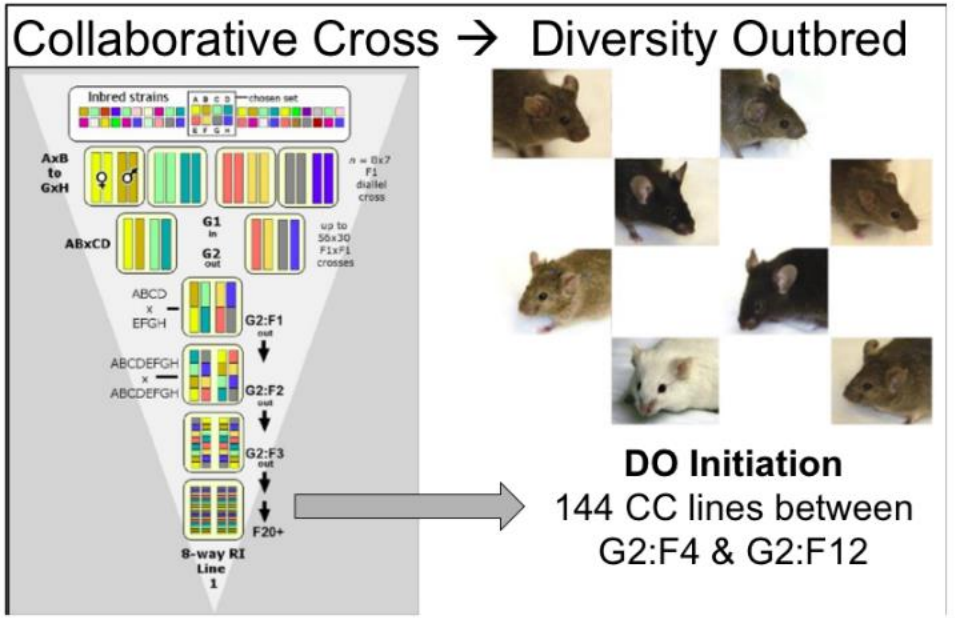
GENETICALLY DIVERSE HUMAN POPULATION

What we're seeking to mimic
Different people > Different genetics

Use a mouse population to inform human susceptibility to chemicals

COLLABORATIVE CROSS AND DIVERSITY OUTBRED

Strain	Letter	Color
A/J	A	
C57BL/6J	B	
129S1/SvImJ	C	
NOD/ShiLtJ	D	
NZO/LtJ	E	
CAST/EiJ	F	
PWK/PhJ	G	
WSB/EiJ	H	



All founder strains have been fully sequenced

Includes representatives of 3 mouse subspecies

- M.m. domesticus
- M.m. musculus
- M.m. castaneous

3 strains were recently derived from the wild

Collaborative Cross

- >100 Recombinant Inbred lines

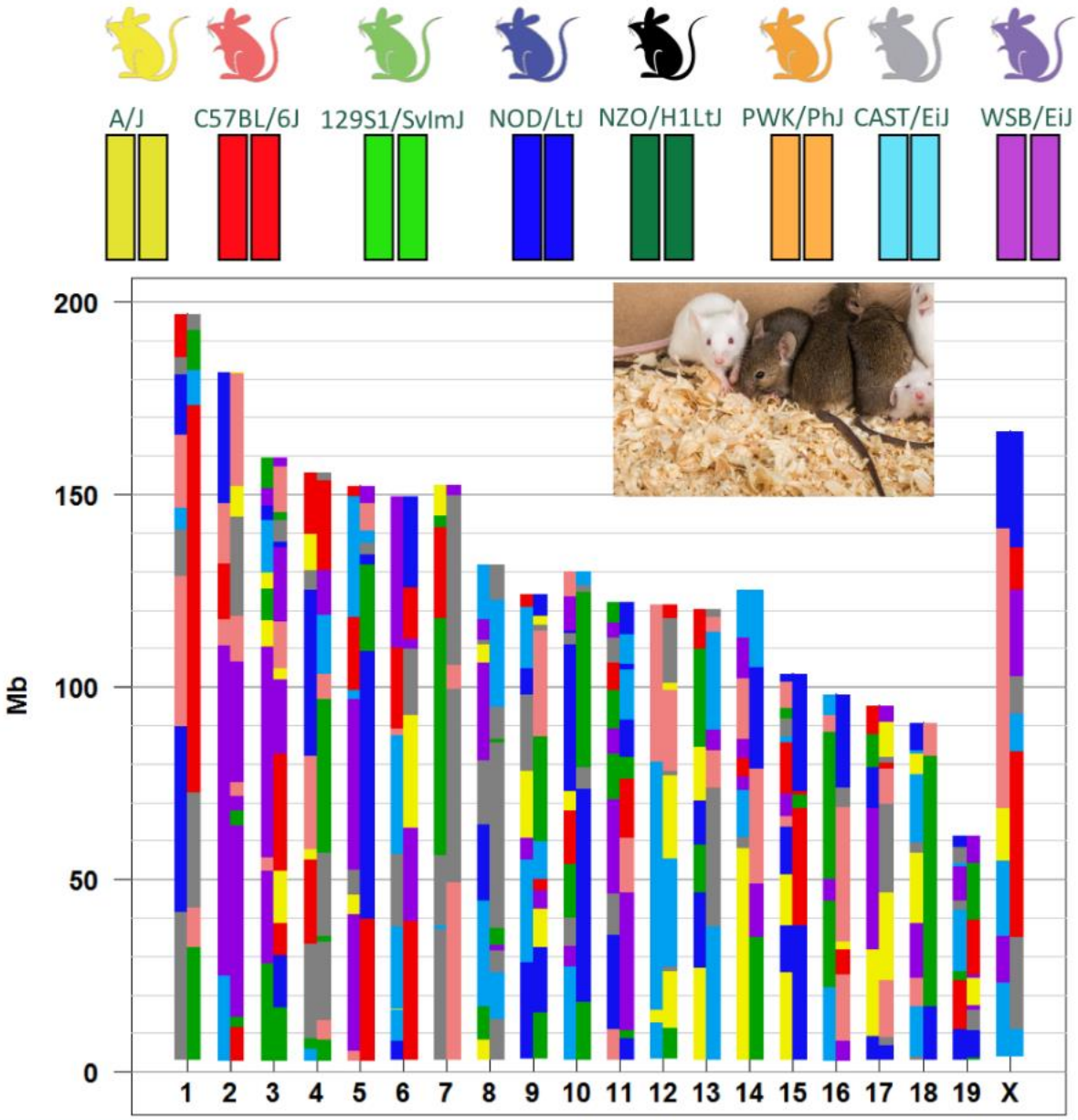
Diversity Outbred Stock

- Each individual is genetically unique
- Sequence mice using MUGA SNP array

An advantage is increased genetic mapping resolution

HUMANS

EXAMPLE DO MOUSE GENOME



OUTBRED MICE

Translational pharmacogenomics

Use mouse populations to predict human population responses

Genome Wide Association Study (GWAS)

AN OVERVIEW

TESTING

Expose diverse mice to chemical and collect samples for quantitative analysis

GWAS

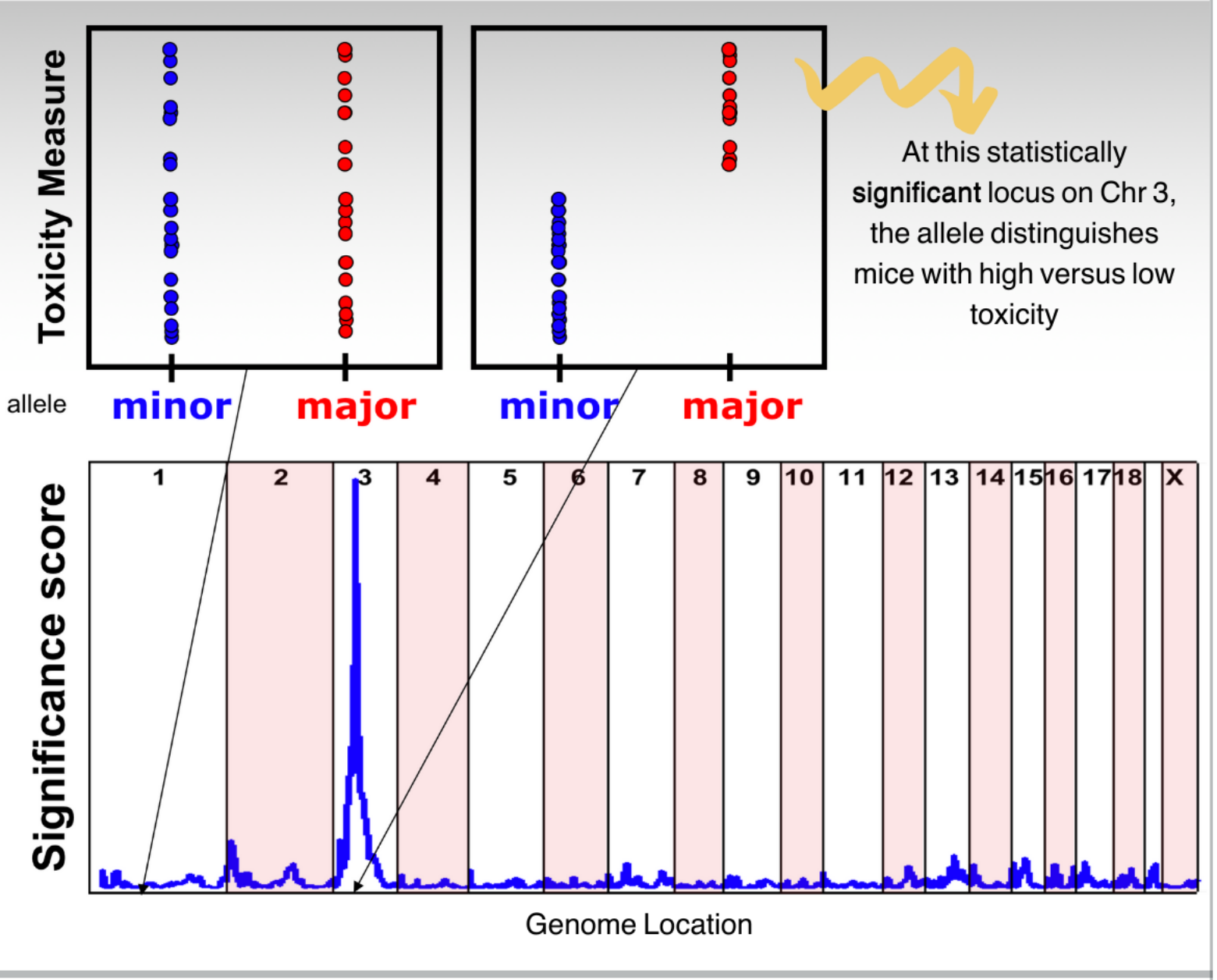
Use statistics to determine regions of genome (loci) that contain variants influencing the trait

SINGLE GENE ANALYSIS

Narrow down candidate gene list, confirm via sequence analysis in mice

TRANSLATION

Sequence candiate genes in humans and confirm association with trait



Manhattan Plot (Example)

ASSESS GENETIC SUSCEPTIBILITY

Basics of GWAS

GWAS is a statistical approach that assesses at each location whether there is a significant difference in the mapped phenotype depending on the locus genotype

It's on Chr 3 that we'll expect to find a pharmacogenetic risk factor for the drug toxicity.

An example of GWAS using Diversity Outbred (DO) Mice

INVESTIGATING GENETIC CAUSE OF RARE, BUT SERIOUS LIVER INJURY THAT OCCURRED DUE TO HERBAL SUPPLEMENTS CONTAINING GREEN TEA EXTRACT

GREEN TEA EXTRACT GWAS

Green Tea Extract

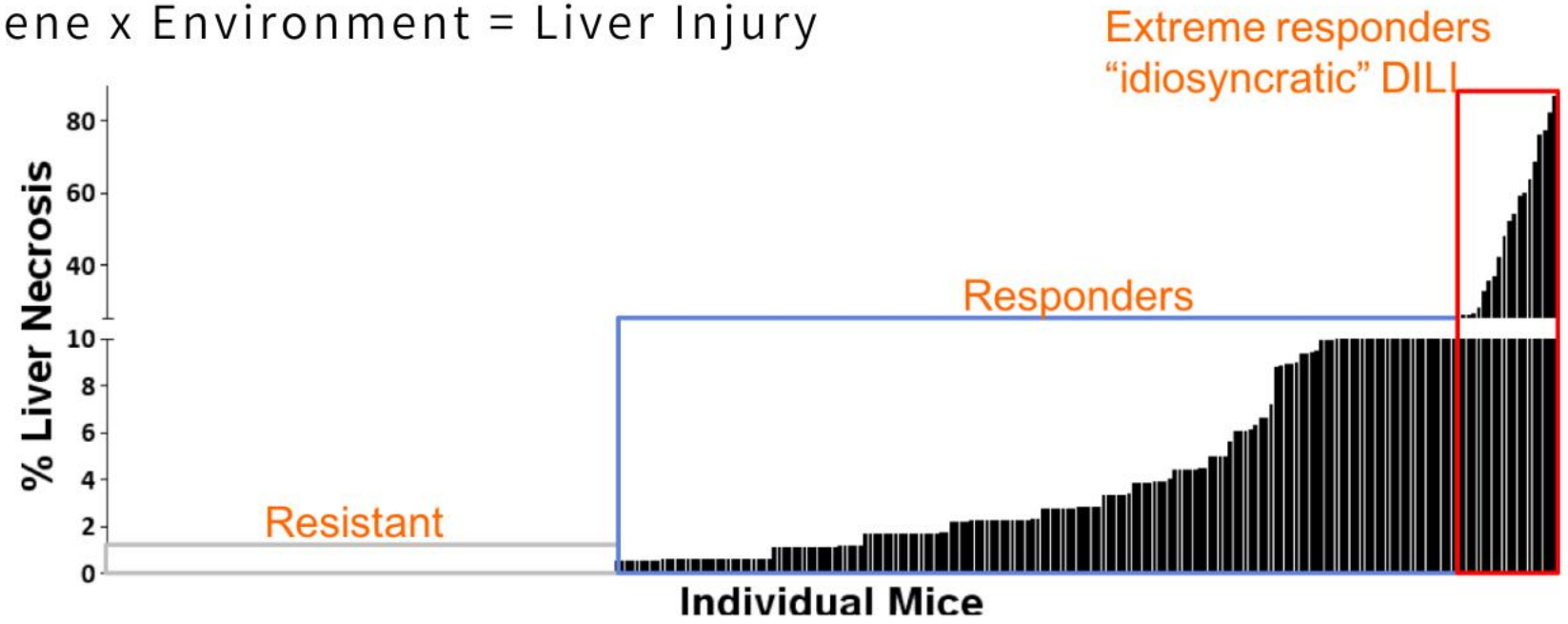
~300 DO mice

50 mg/kg p.o. over 3 days

Church et al. Food and Chem Tox. 2015

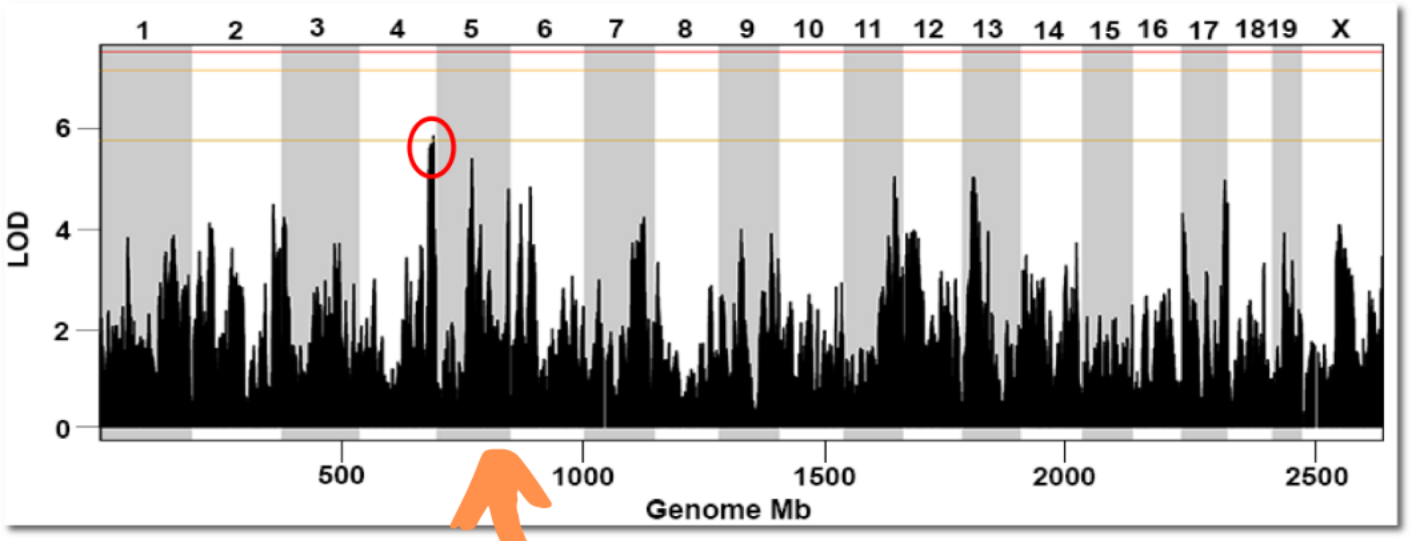
Epigallocatechin gallate

Gene x Environment = Liver Injury

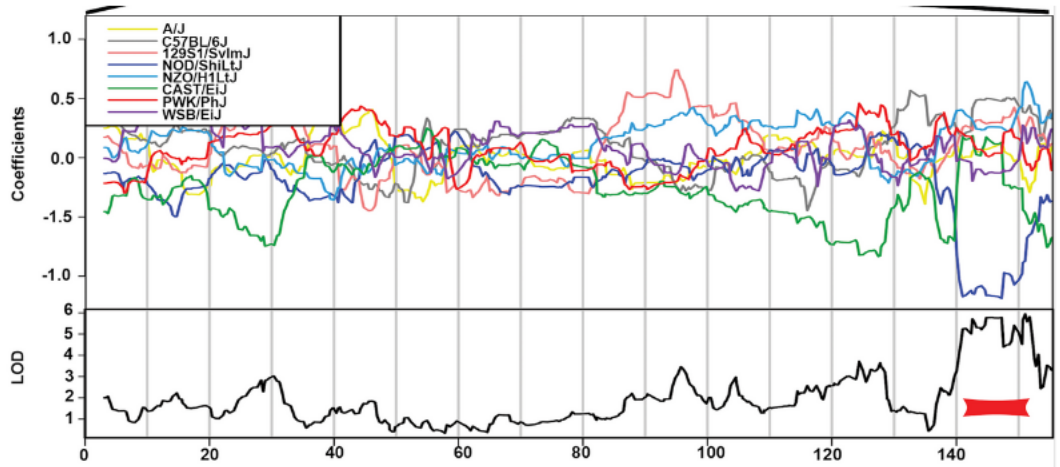


A small fraction of the animals (16%; 43/272) exhibited severe hepatotoxicity (10-86.8% liver necrosis) that is analogous to the clinical cases

DO GWAS - Green Tea Extract



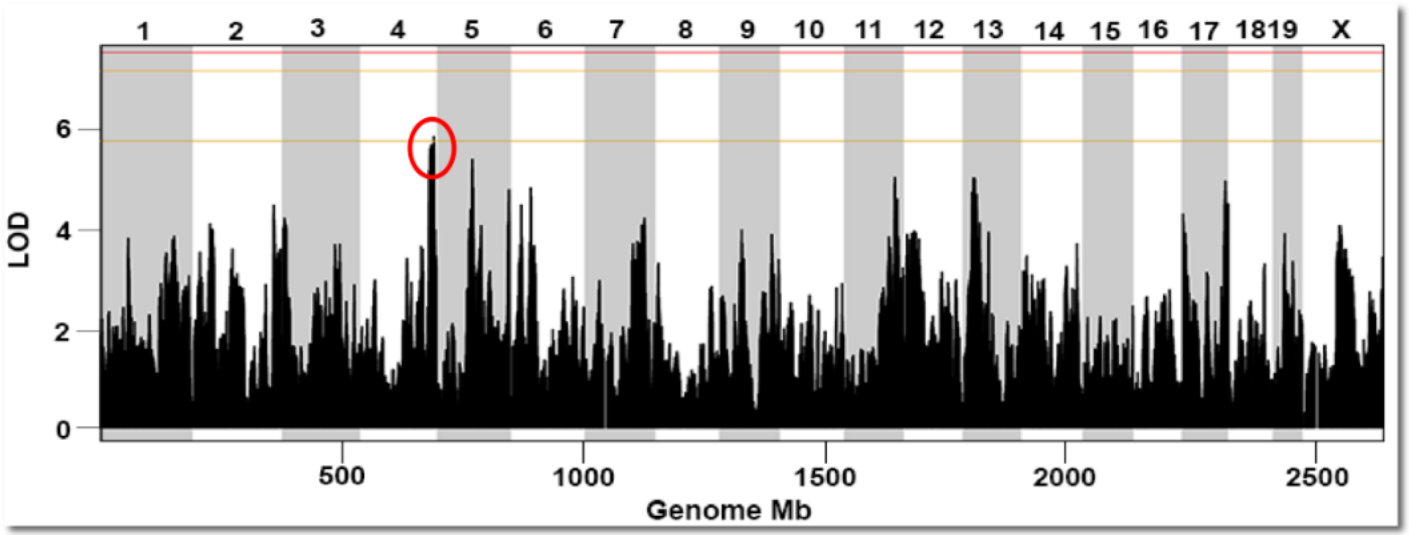
For green tea extract, there was a significant peak on the distal arm of Chr 4

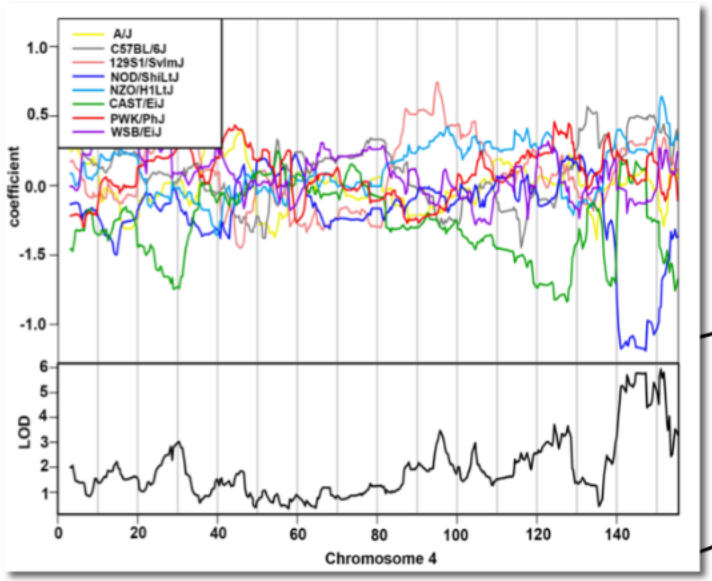


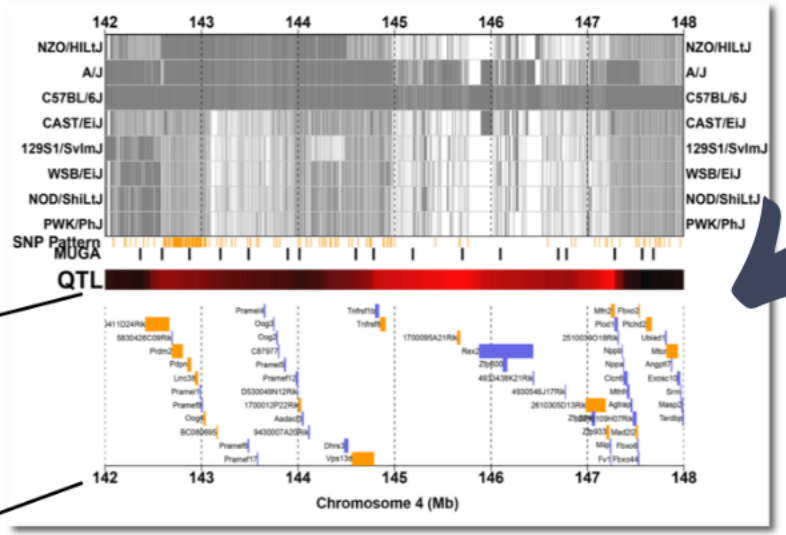
We can expand the significant gene region

Because we have the complete sequence and haplotype structure of each DO mouse, can determine what is the effect on the phenotype of having inherited that DNA "chunk" from one of the 8 founders

DO GWAS - Green Tea Extract



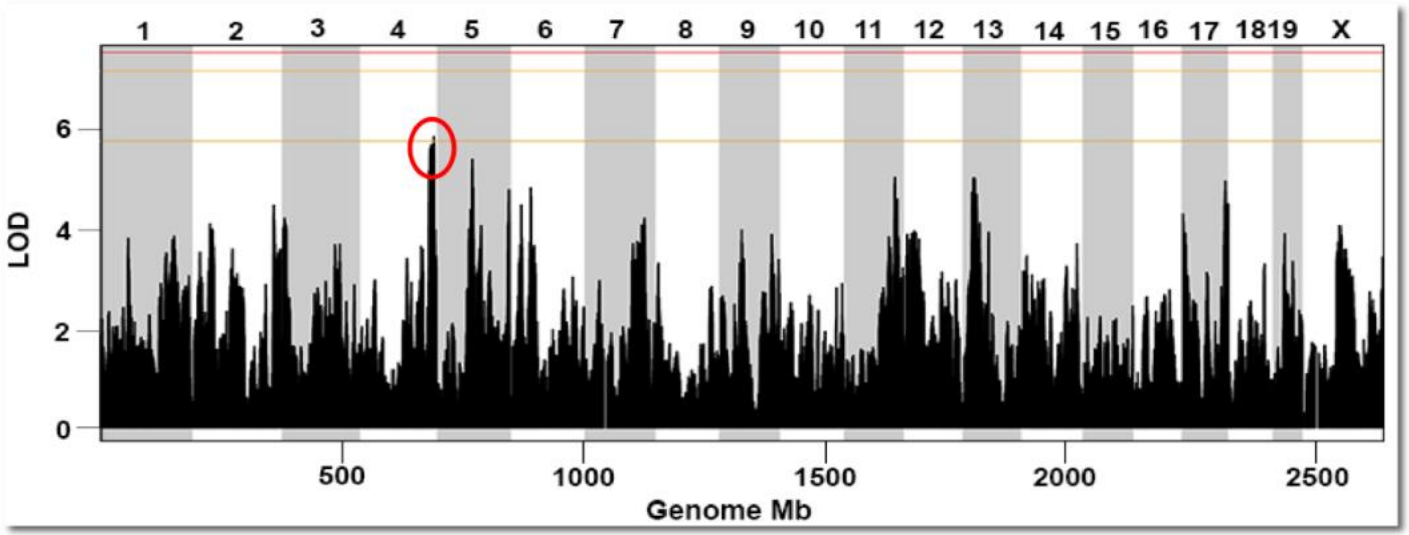




We can use the founder effects to narrow down the list of quantitative trait genes in the QTL support interval

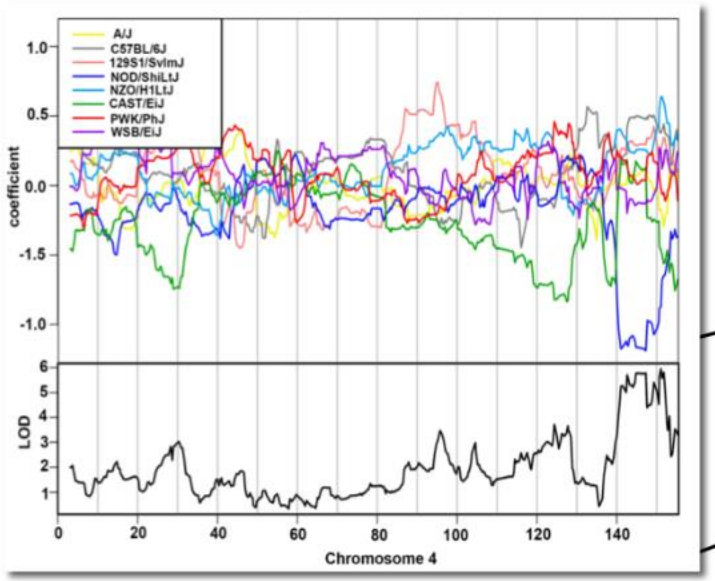
Focus targeted sequencing on just those genes that contain a unique NOD founder allele

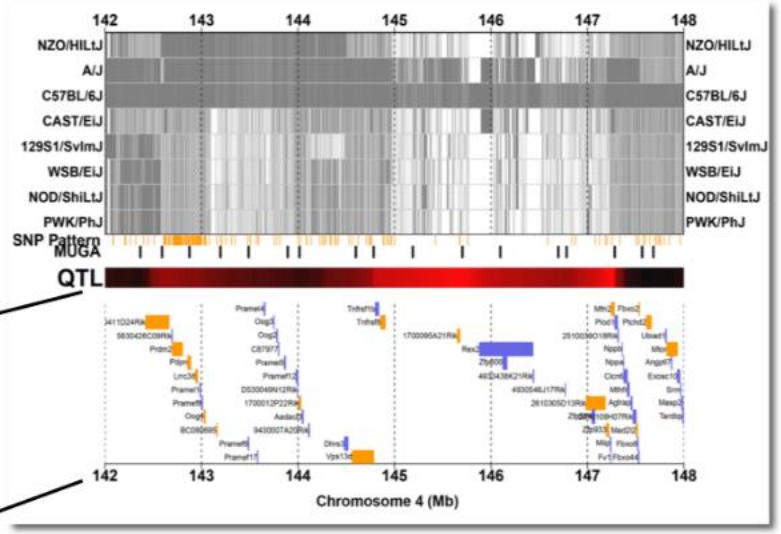
DO GWAS - Green Tea Extract



Sequencing candidate genes in 15 clinical cases of green tea extract induced liver injury

Gene Symbol	SNP ID (Array)	Gene Name	Chromosome	Position	P value for clinical association	Risk/Protective allele	Effect
PER3	exm10762	period circadian clock 3	1	7887234	0.004937	T/C	Missense (R/W)
MFN2	exm15928	mitofusin 2	1	12069692	0.0067	A/G	Missense (I/V)
VPS13D	exm16480	vacuolar protein sorting 13 homolog D (S. cerevisiae)	1	12343493	0.043064	A/T	Missense (R/S)





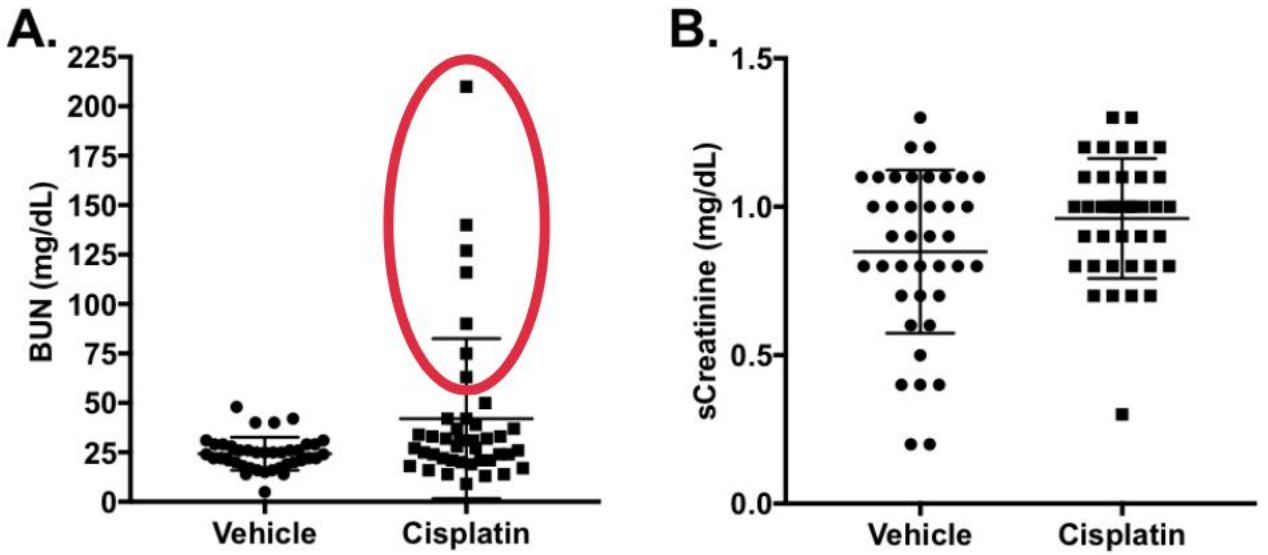
Mitofusin 2, involved in mitochondrial regulation and maintenance, may contribute to susceptibility to EGCG-induced liver injury by herbal supplement use.

Investigating gene by environment for chemotherapeutic toxicity

Once you've identified susceptible and resistant mice - mechanistic and biomarker analysis becomes tractable

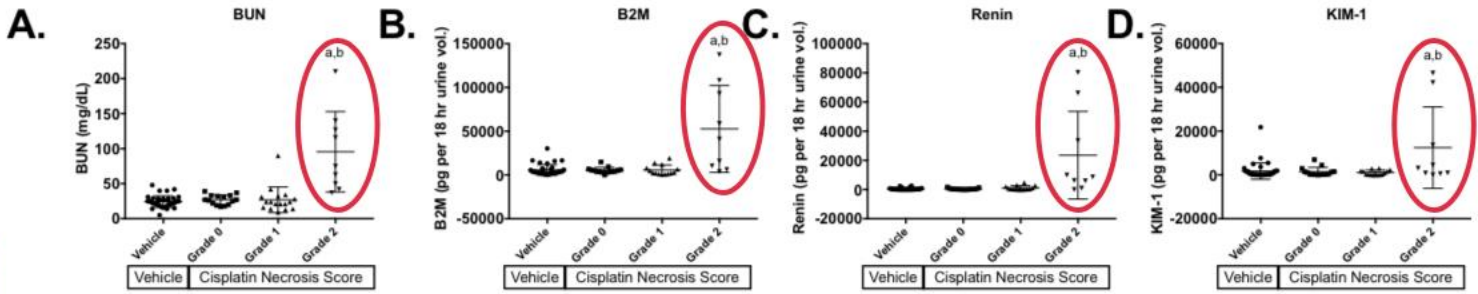
Gene x Chemotherapeutic Drug Interactions

Differential kidney toxicity responses in DO mice due to chemotherapeutic cisplatin



MARKERS OF KIDNEY INJURY ELEVATED

5 mg/kg cisplatin i.p.; 3 days after exposure



Urinary biomarkers of proximal tubule injury elevated only in susceptible DO mice

Harrill et al. Exp Biol Medicine 2018

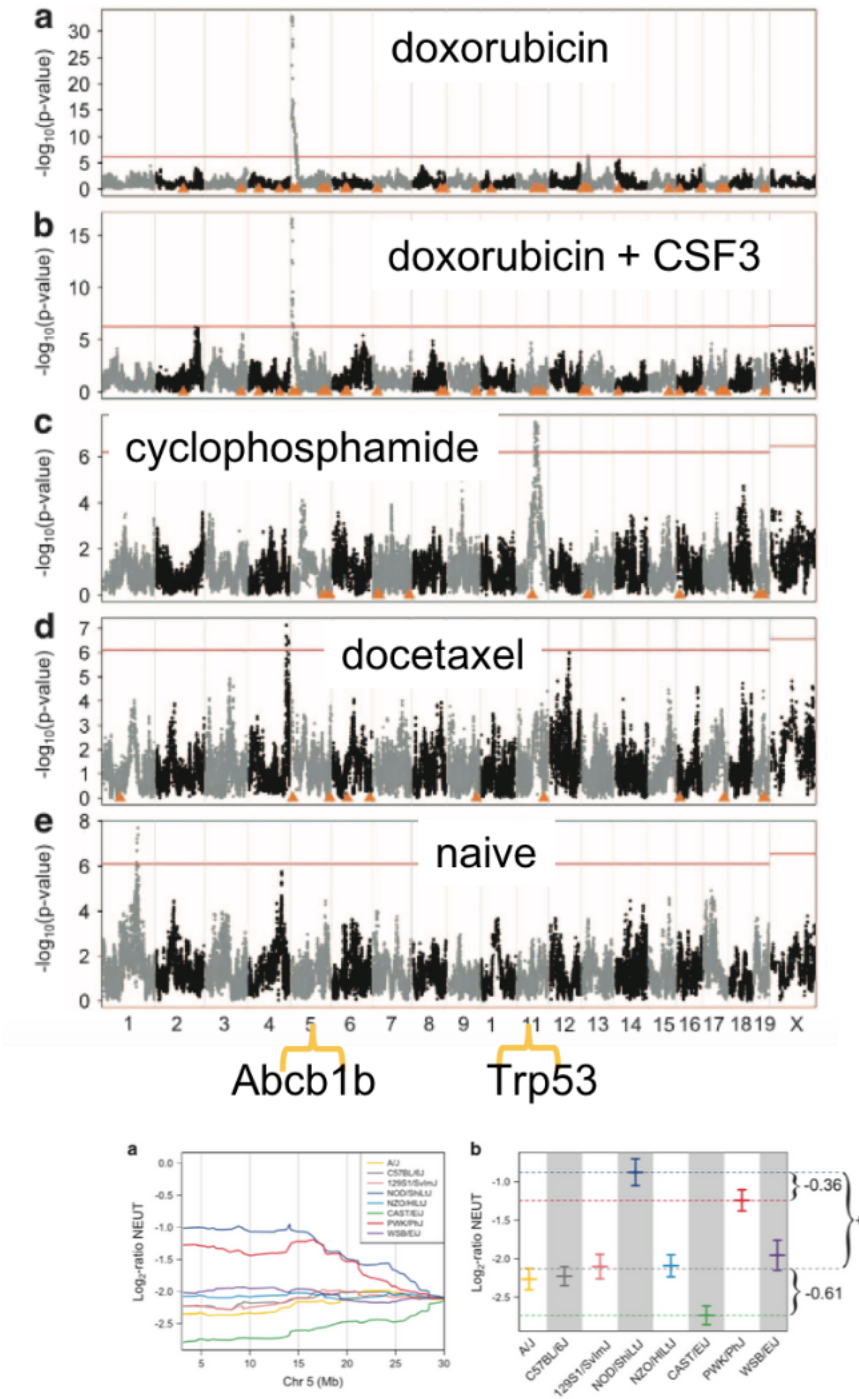
Myelosuppression caused by chemotherapy

GENETIC SUSCEPTIBILITY

Investigation of genetic variants in DO mice that increase susceptibility to decreased neutrophil counts in response to:

doxorubicin
cyclophosphamide
docetaxel

Gatti et al. Pharmacogenomics J 2018



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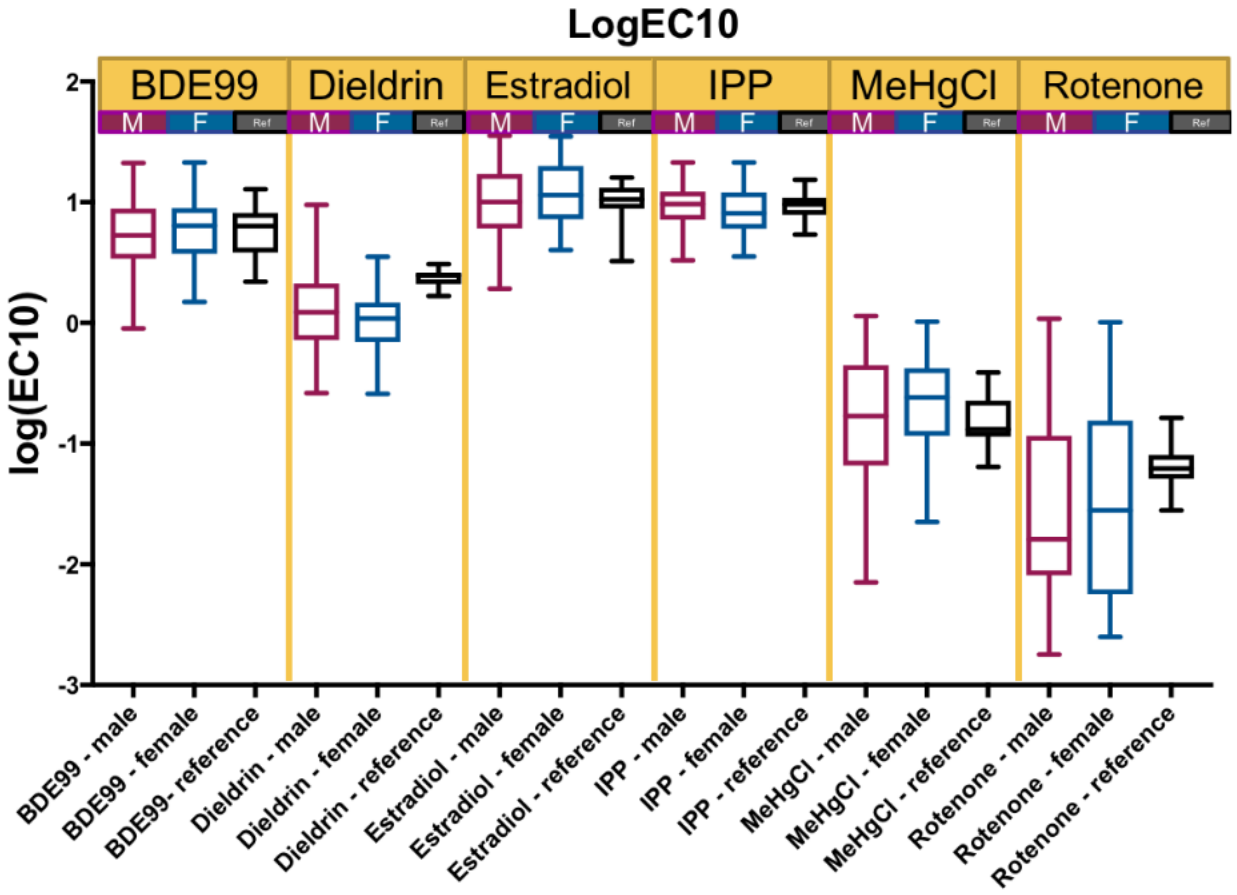
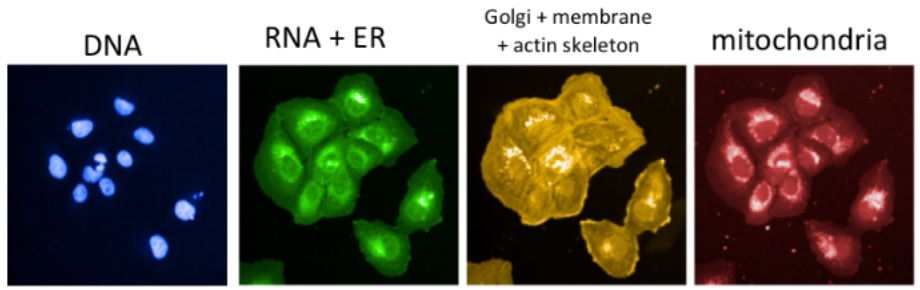
DO IN VITRO EXPERIMENTS - DNT



Cell Painting



Effect of drugs and chemicals on brain development of offspring

Emphasis on developmental brain injury in 200 male and female Diversity Outbred neural progenitor cell lines

Including genetic diversity in toxicology and in disease modeling can improve understanding and prediction of human outcomes, enabling precision medicine

HUMAN HEALTH PROTECTION

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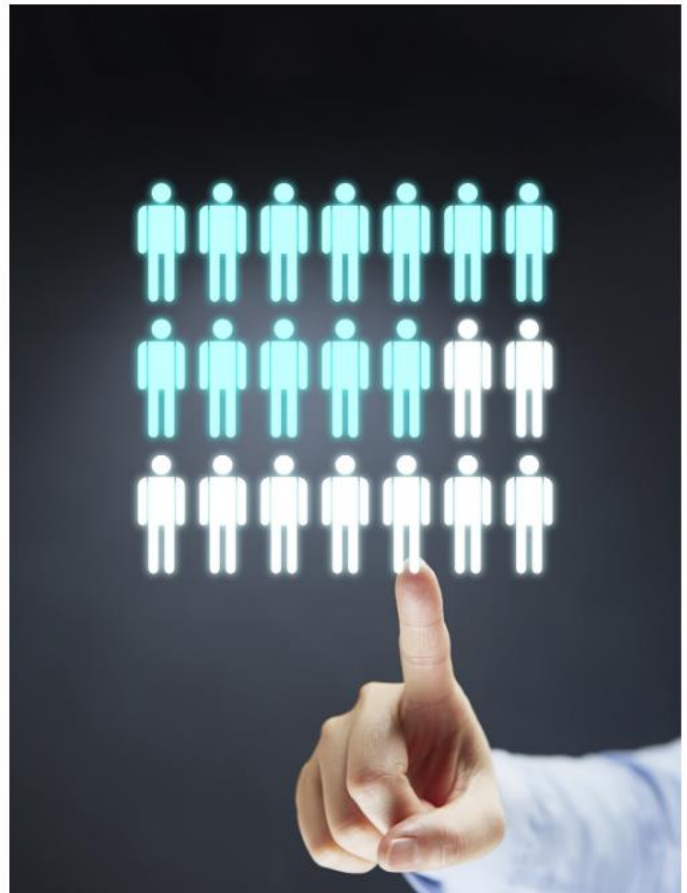
Predictive Biology



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THANK YOU FOR YOUR ATTENTION!