The Mouse Diversity Panel Predicts Clinical Drug Toxicity Risk Where Classical Models Fail

Alison Harrill, Ph.D

The Hamner-UNC Institute for Drug Safety Sciences

THE HAMNER INSTITUTES FOR HEALTH SCIENCES

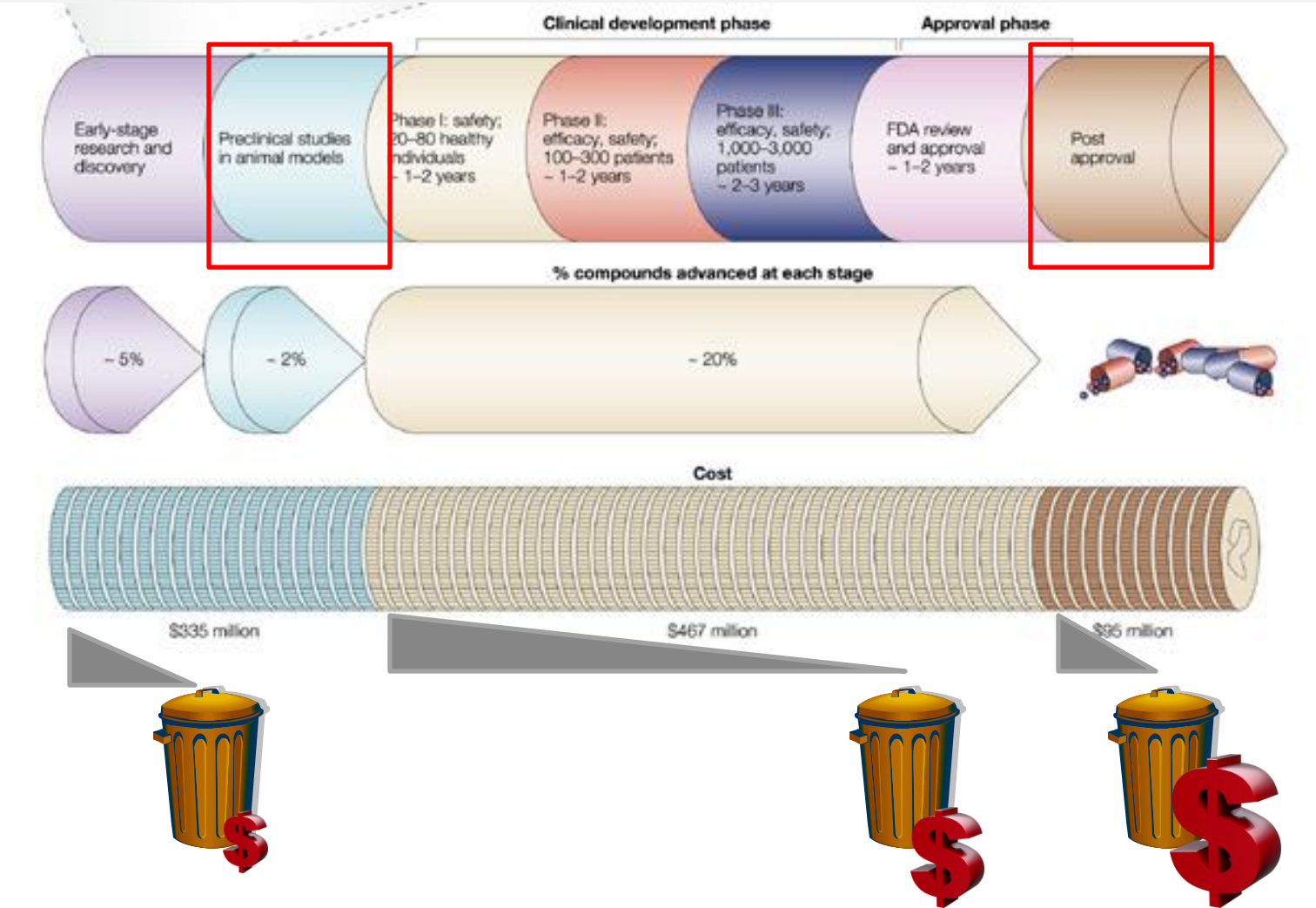
THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

The Importance of Predicting Clinical Adverse Drug Reactions (ADR)

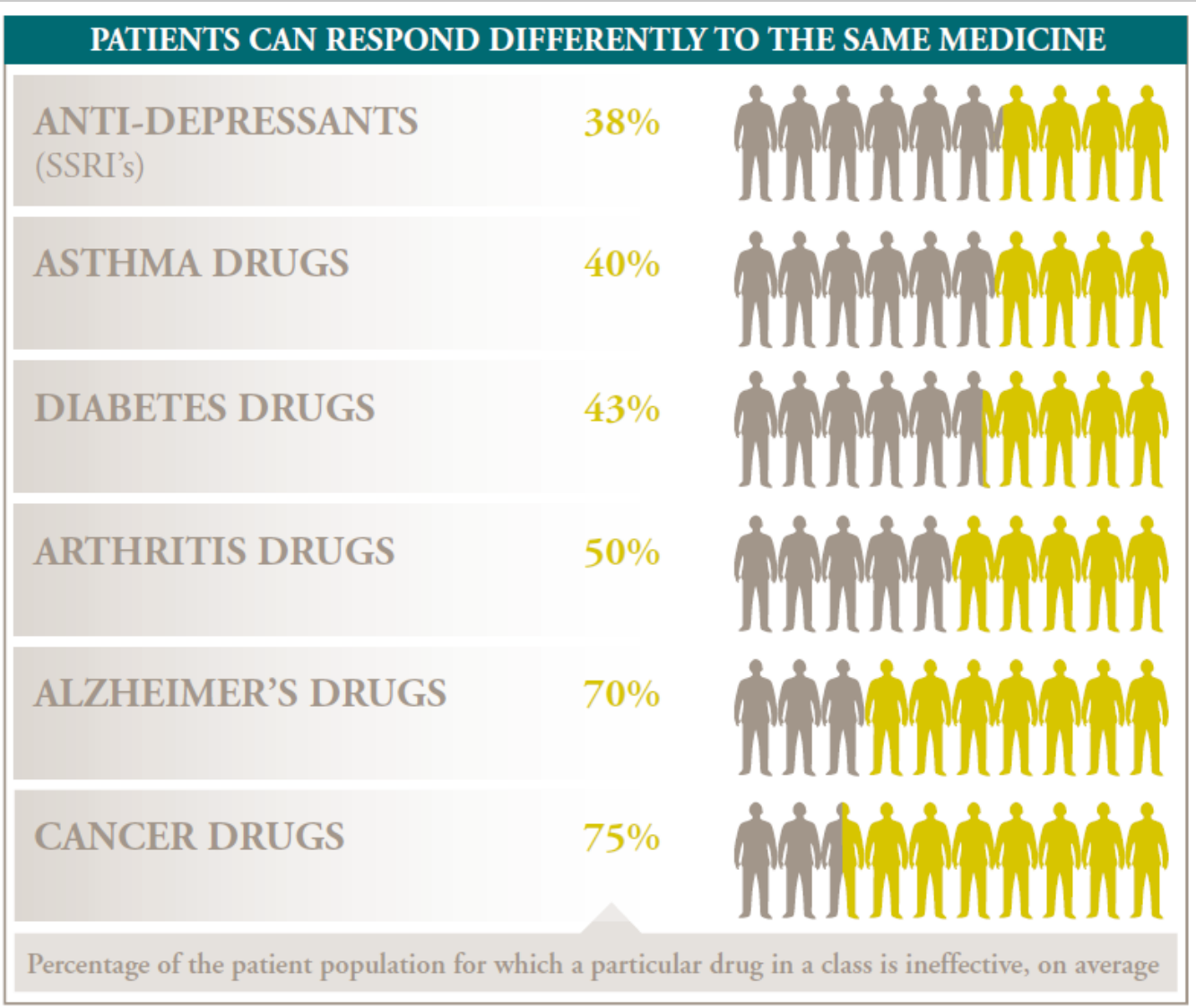
Figure: Cath O'Driscoll Nature Publishing 2004

Risk ID

PGx Testing



People Respond Differently to Drugs



Source of data: Brian B. Spear, Margo Heath-Chiozzi, Jeffrey Huff, "Clinical Trends in Molecular Medicine, Volume 7, Issue 5, 1 May 2001, Pages 201-204.

Pharmacogenetic Markers Identified by Genome-Wide Association

Drug	Adverse Drug Reaction (ADR)	Risk Allele
Abacavir Flucloxacillin	Hypersensitivity Hepatotoxicity	HLA-B*5701
Allopurinol	Cutaneous ADR	HLA-B*5801
Carbamazepine	Stevens-Johnson Syndrome	HLA-B*1502
Augmentin	Hepatotoxicity	DRB1*1501
Ximelagatran	Hepatotoxicity	DRB1*0701
Ticlopidine	Hepatotoxicity	HLA-A*3303

Average preclinical populations and human hepatocytes lack the diversity to detect incidence of adverse events that occur only in 1/10,000 people.

Current Rodent Models of Risk Assessment
The Challenge

"At a time of extraordinary scientific progress, methods have hardly changed in several decades ([FDA] 2004)… Toxicologists face a major challenge in the twenty-first century.

They need to embrace the new "omics" techniques and ensure that they are using the most appropriate animals if their discipline is to become a more effective tool in drug development."

-Dr. Michael Festing

Quantitative geneticist

Toxicol Pathol. 2010;38(5):681-90

Rodent Models as a Strategy for Hazard Characterization and Pharmacogenetics

Genetically defined rodent models may provide ability to:

1. Improve preclinical prediction of drugs that carry a human safety risk

2. Identify genetic factors that predict an individual patient's risk (or benefit), thereby:

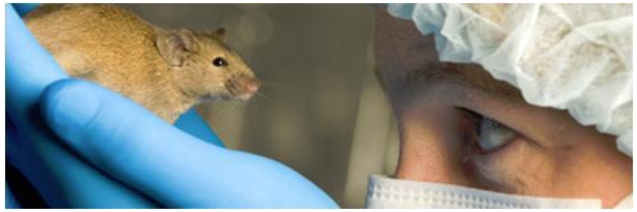
    1. Allowing otherwise efficacious drugs to remain on the market
    2. Providing insight into mechanisms that guide design of next-in-class compounds

Key Aspects of Translational Pharmacogenetics Using Mice

- Models allow for controlled experiments
  - Variables (ex.): timing and dose of drug administration, diet, environment, genetic variation
  - Better prediction of toxicity

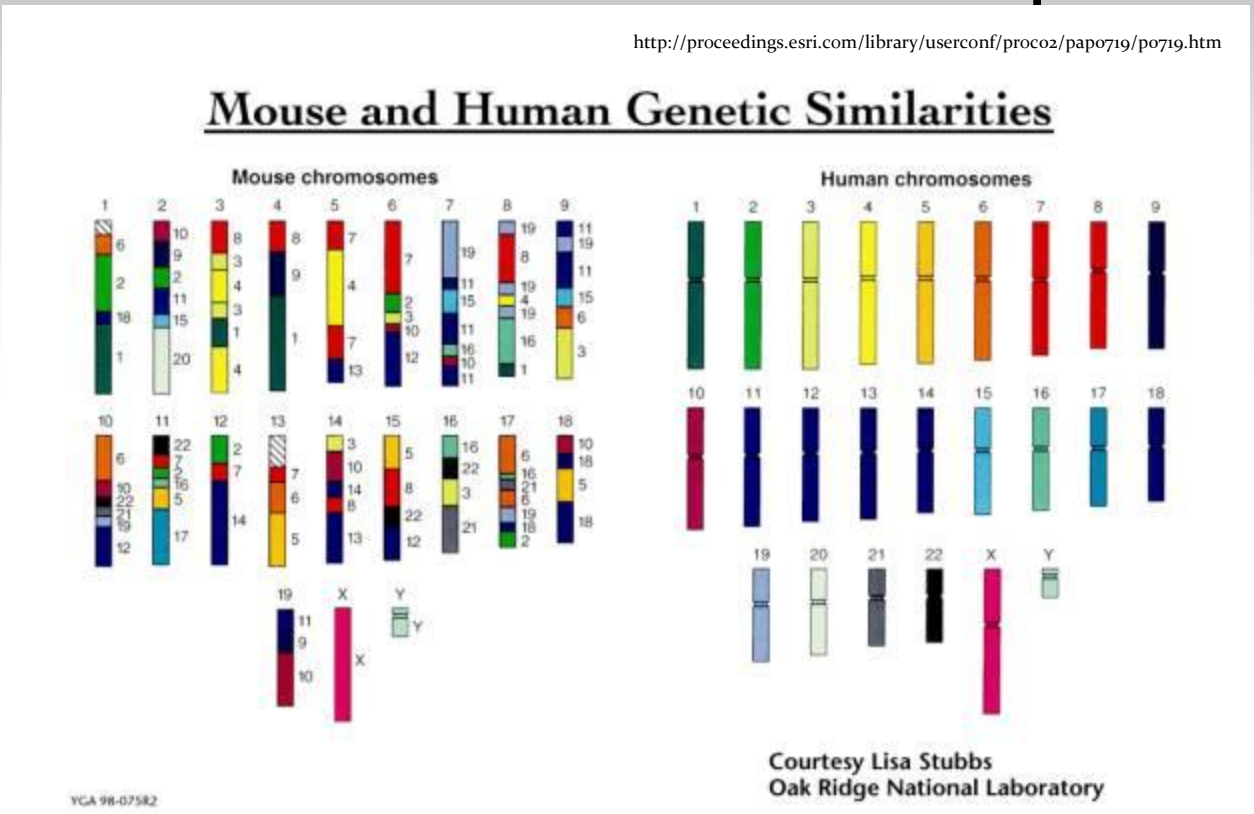
- Experiments can be replicated
  - Think of well-characterized animals as a “defined reagent”

- Ethical concerns might preclude treating humans with a compound known to be toxic, but for which the mechanisms remain to be elucidated



Mouse to Human Genetic Comparison

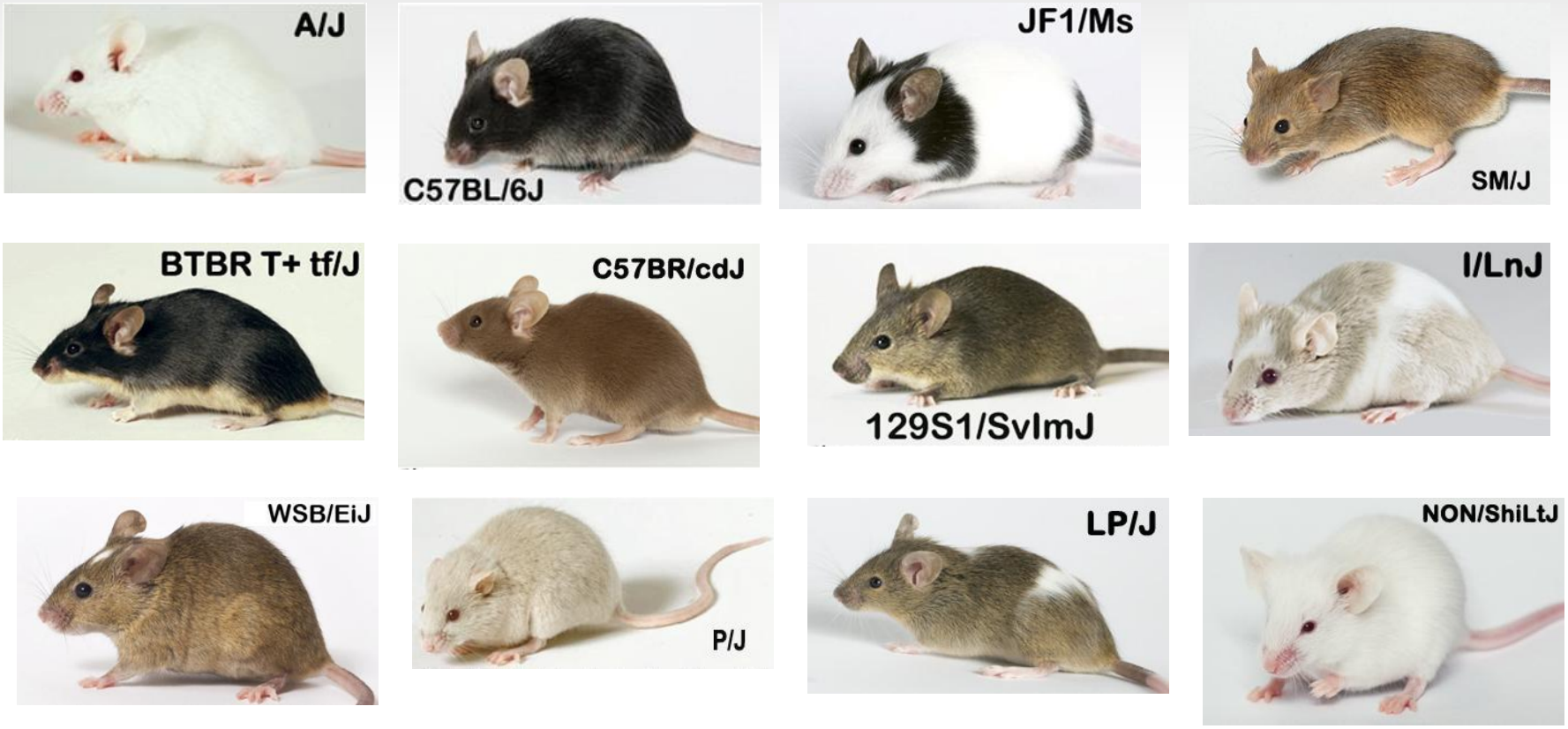
http://proceedings.esri.com/library/userconf/proc02/pap0719/p0719.htm



- Gene order of the genomes in mice and humans are largely conserved (synteny)
  - Although, there are rearrangements, several per chromosome in mouse

- The mouse to the rescue?

Hundreds of Mouse Stains Available



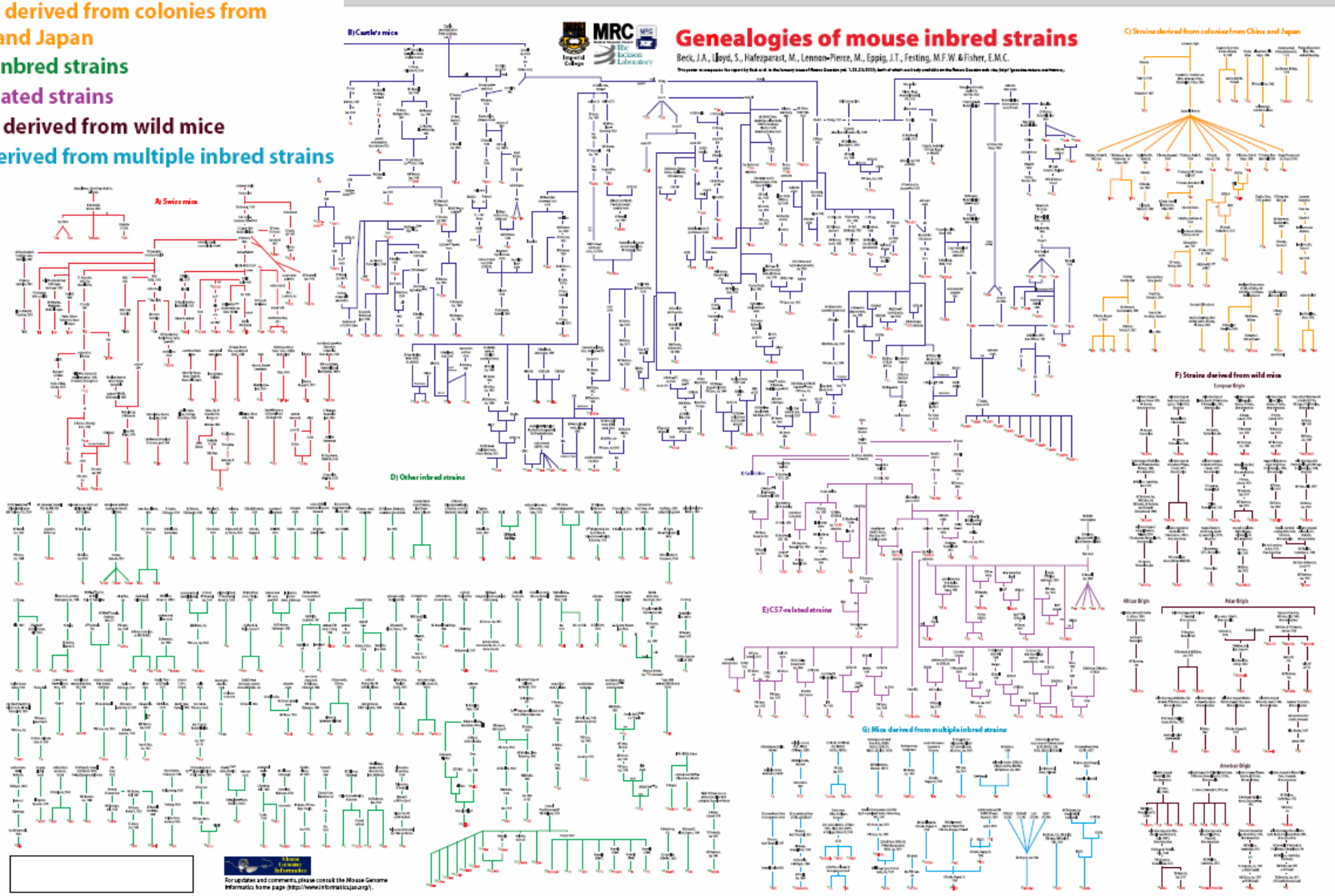
Photos by Stanton Short, Jackson Laboratory

Wide variation in toxicity response, behavior, exercise patterns, glucose tolerance, cancer susceptibility, coat color, weight, etc…

Many Inbred Strains Available

Inbred strain categories

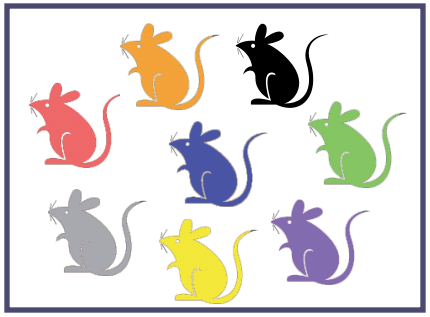
- A) Swiss mice
- B) Castle's mice
- C) Strains derived from colonies from China and Japan
- D) Other inbred strains
- E) C57-related strains
- F) Strains derived from wild mice
- G) Mice derived from multiple inbred strains



Beck *et al. Nature Genetics* 2000.

Using Inbred Mouse Panels to Identify Genes Associated with Toxicity

- Mouse Diversity Panels (MDP): Harness a great deal of genotypic diversity



Genetically Diverse Mouse Population

"Mouse Diversity Panel"
Classical Inbred Mouse Strains

Pros:
1) Only need to be genotyped once
2) Repeat testing within a single strain for better mean estimate
3) Extensive genetic polymorphism (SNP) databases available

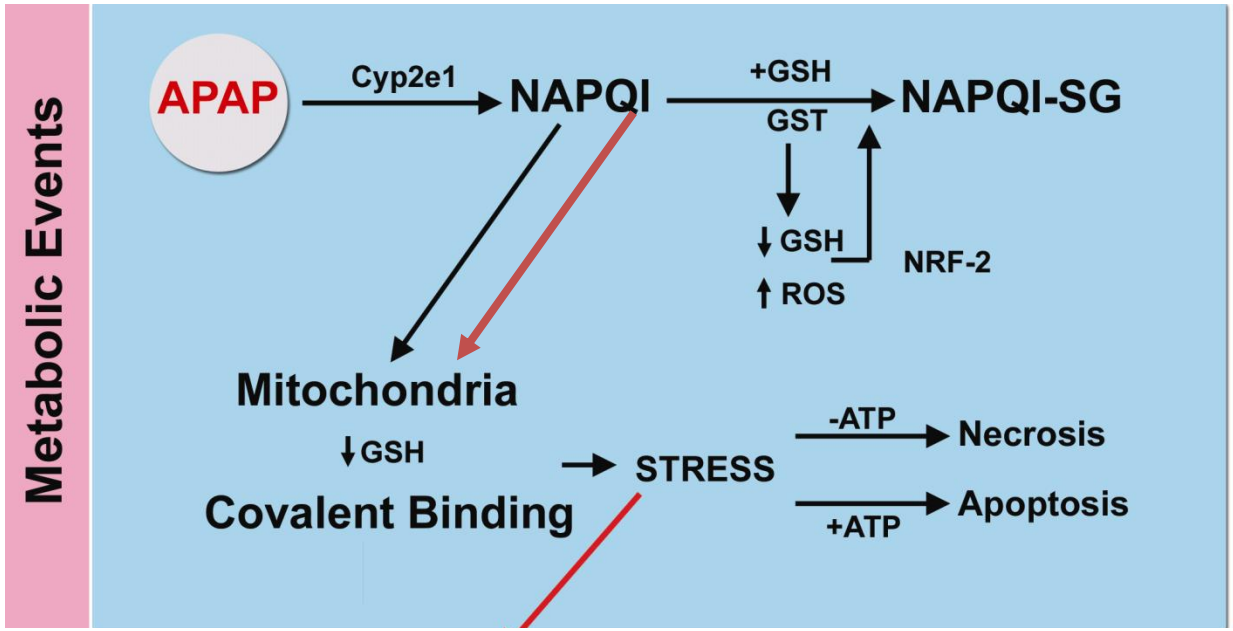
Genetically Diverse Human Population

Genetic diversity across mouse strains comparable to human populations

Acetaminophen as a Model Liver Toxicant

- Acetaminophen (APAP; Tylenol®) as a model compound
  - Considered a dose-dependent liver toxicant
  - Well-characterized hepatotoxic phenotype after overdose:
    - Centrilobular necrosis
    - Increase in serum alanine aminotransferase (ALT)

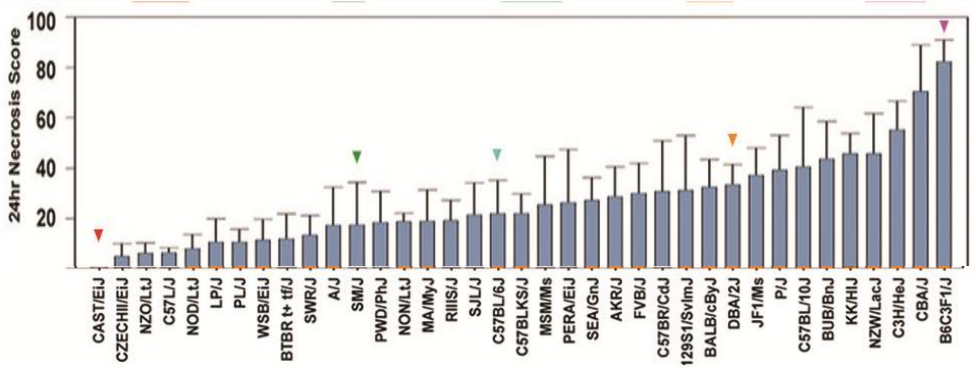
- In U.S. and many other countries, APAP overdose is the leading cause of liver injury due to a pharmaceutical agent
  - Intentional overdose
  - Accidental overdose - “therapeutic misadventure”
  - Taken after alcohol ingestion (increases reactive metabolite formation)
  - Idiosyncracy – DILI at apparent therapeutic doses



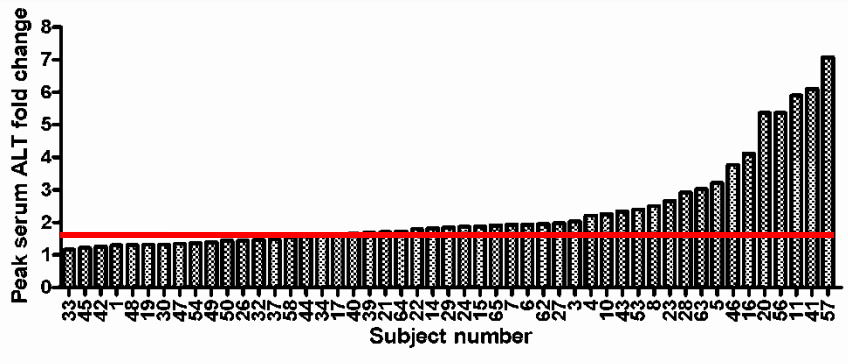
Schematic adapted from Kaplowitz, N. *Nat. Rev. Drug Discov.* **4**, 489-499 (2005)

Acetaminophen Toxicity in Mouse and Human Populations

Mouse Phenotypic Responses



Human Phenotypic Responses



Mouse APAP Exposure:

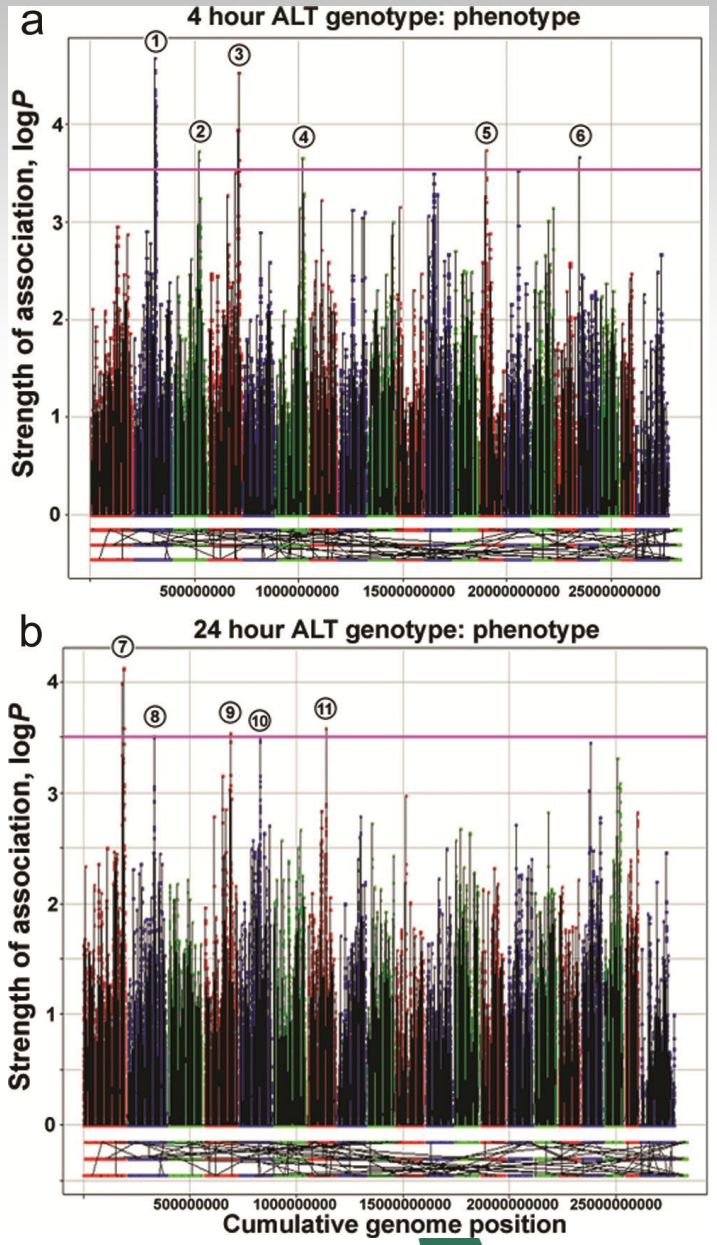
- 300 mg/kg in 36 inbred mouse strains
- Males
- Singly caged
- Variation in liver necrosis by genotype

Human APAP Exposure:

- 50 mg/kg day for 7 days (dose every 6 hr)
- 69% of subjects had significant toxicity response
- Subjects in-clinic, std. diet
- Healthy volunteers

Harrill, AH *et al. Genome Res.* 2009.

Haplotype-Associated Mapping



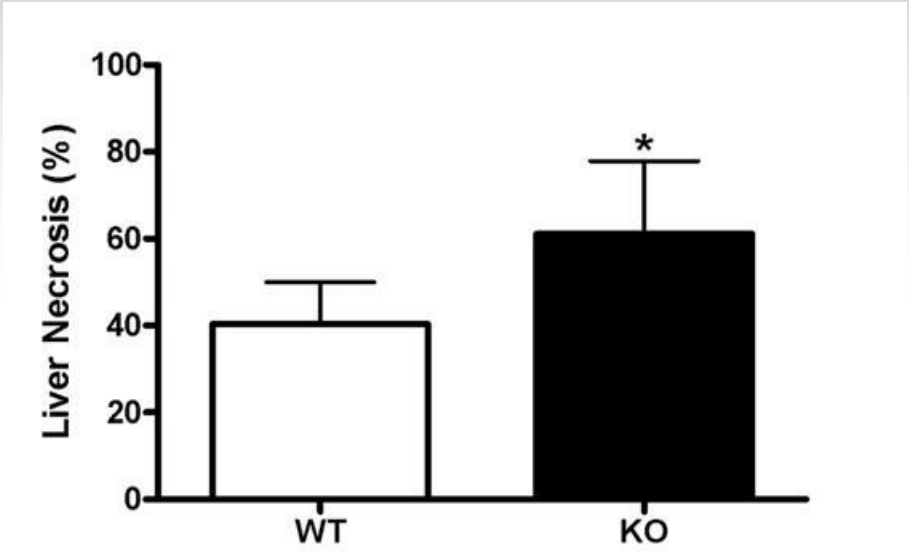
Genome-wide association mapping

Phenotype	Peak	Genome position (Mb)	Genes in region
4 Hr ALT	1	Chr 2: 102.08-106.96	*Trim44, E430002G05Rik, Slc1a2,* ***Cd44****, Pdhx, Apip, Ehf, BC016548, Elf5,* ***Cat****, Abtb2, Nat10, Gpiap1, Lmo2, 4931422A03Rik, Fbxo3, Cd59b,* ***Cd59a****, A930018P22Rik, D430041D05Rik, Hipk3, Cstf3, Tcp11l1, AV216087, Qser1, Prrg4, Ccdc73, Ga17, Wt1, 0610012H03Rik , Rcn1, Pax6, Elp4, Immp1l, Zcsl3, 4732421G10Rik, Mpped2, 2700007P21Rik, Fshb*
	2	Chr 3: 126.439–26.844	*Ank2*
	3	Chr 4: 141.531–43.578	*Prdm2, Pdpn, Lrrc38, Pramel1, 4732496O08Rik, Oog4, BC080695, Pramel5, Pramel4, Oog3*
	4	Chr 6: 123.795–24.766	*V2r1b, Cd163, Pex5, Clstn3, C1rl, C1r, Oact5, Emg1, Phb2, Ptpn6, Grcc10, Atn1*
	5	Chr 13: 36.862–37.022	***Ly86***
	6	Chr 17: 5.598–5.655	*Zdhhc14*
24 Hr ALT	7	Chr 1: 182.602–82.719	***Capn8***
		Chr 1: 189.550–89.735	*Prox1*
	8	Chr 2: 127.489–27.580	*Bub1, 1500011K16Rik*
	9	Chr 4: 124.084–24.395	*Utp11l, Fhl3, Sf3a3, Inpp5b, Mtf1, Yrdc, Gm50, Epha10, Cdca8, 9930104L06Rik*
	10	Chr 5: 97.392–97.681	*Prdm8, Fgf5, 1700007G11Rik*
	11	Chr 7: 86.492–86.594	No known genes

Result: Significant association with *Ly86, Cd44, Cd59a, Capn8* determined by resequencing

Harrill, AH *et al. Genome Res.* 2009.

CD44 Polymorphism Functional Data in Mice

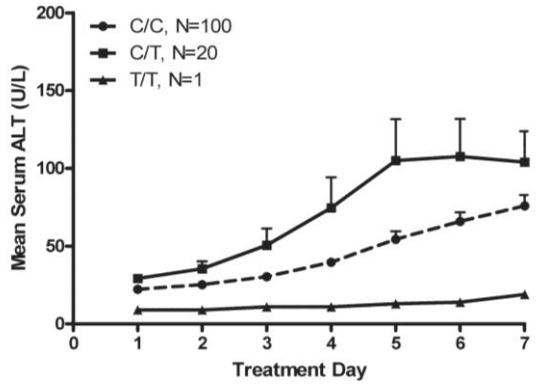


- Mice: C57BL6/J WT or KO mice (N=6/group)
- Dose: 300 mg/kg APAP after overnight fast
- Necropsy: 24 hr post-dose

RESULT: *Cd44* KO mice have a greater toxicity outcome than wild type

Human Polymorphisms Affect Toxicity Outcome -- *CD44*

UNC + Purdue Pharma

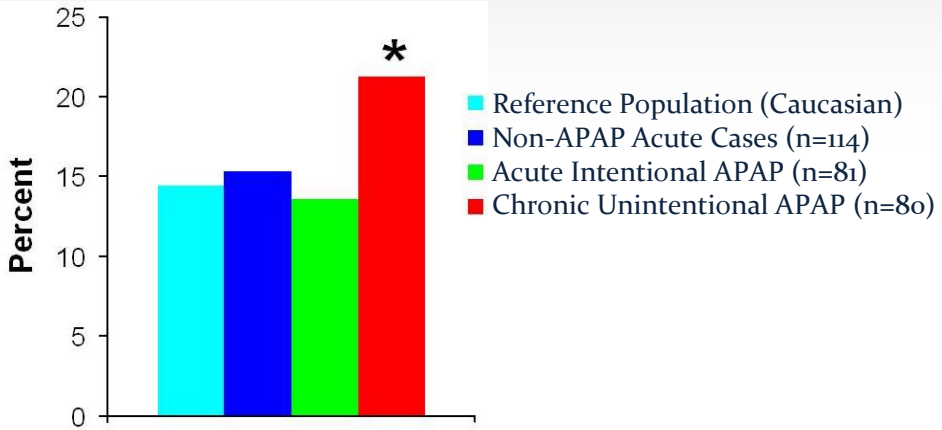


UNC + Purdue Pharma Study Population

121 APAP subjects

50 mg/kg (2 tablets/6hr)

CD44 Polymorphism Allele Frequency in 275 Caucasian Acute Liver Failure Subjects



Unpublished data from an NIDDK Acute Liver Failure Study Group ancillary study

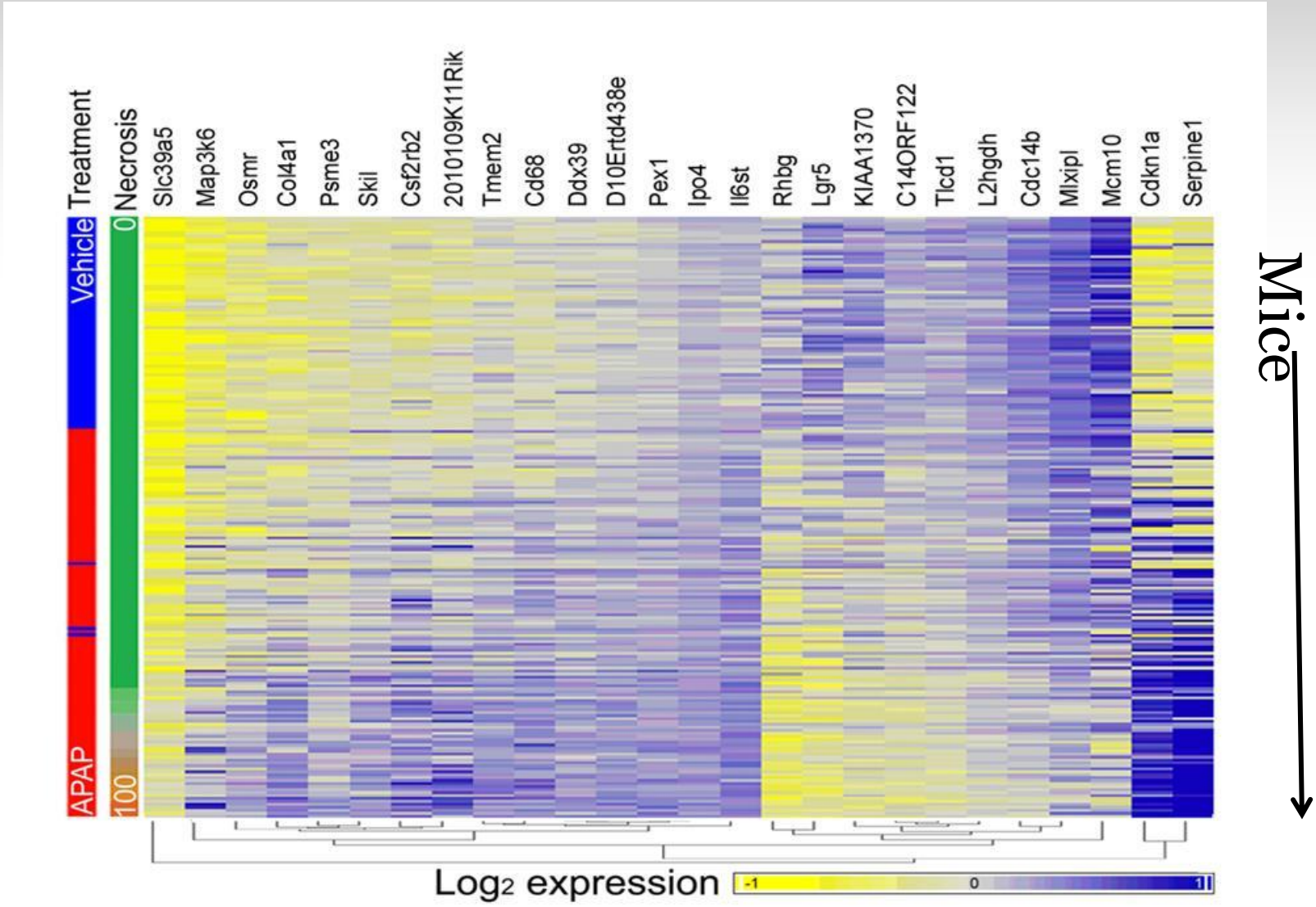
P.I. – Michael Court (Tufts U.)
Co-P.I. – W.M. Lee (UTSW)

* P = 0.014 by Chi-squared test

Harrill, AH *et al. Genome Res*. 2009.

Will Lee and Michael Court, Unpublished data used with permission

26 Population-Based mRNA Biomarkers of APAP-Induced Liver Injury

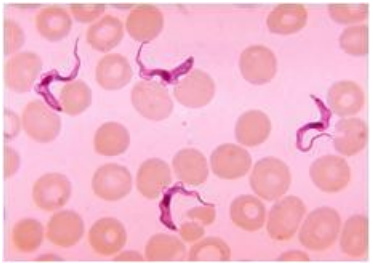


DB289 - A Drug that Failed Clinical Trials

- DB289 – promising new drug to treat infection of trypanosomal parasites (sleeping sickness), a fatal disease endemic to sub-Saharan Africa

Case Study: DB289 for HAT

- DB289 was oral pro-drug for Human African Trypanosomiasis (sleeping sickness) treatment
  - Vector borne parasite transmitted by tsetse fly in rural Africa
- First stage treatment (pentamidine; 60 years) associated with liver tox, hypotension
- Second stage treatment (melarsoprol) associated with encephalopathy in 5-10%
  - Of whom 10-50% cases will be fatal



CPDD
Consortium FOR
Parasitic Drug Development

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

DB289 Clinical Trials Efficacy

- Phase II
  - Democratic Republic of Congo (DRC), Angola
  - 93% cure rate at 3 mo. post-treatment
  - No safety concerns
- Phase III
  - DRC, Angola, Sudan
  - Hepatotoxicity of DB289 <<< pentamidine
    - (7 vs. 77%)
  - 84% cure rate 24 mo. post-treatment

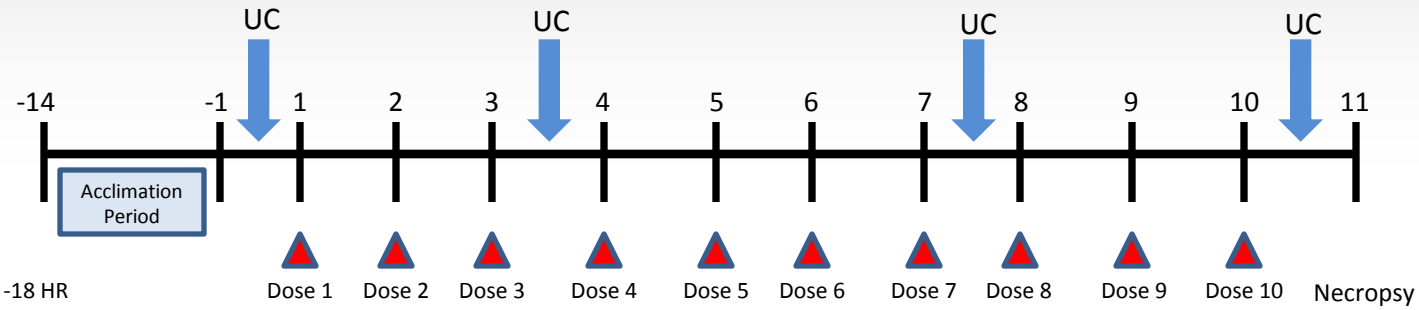
DB289 Clinical Trials Expanded Phase I Safety

- Conducted in South Africa

- Frequent hepatobiliary adverse events put study on hold
  - 28 volunteers (35%) with ALT ≥ 3X ULN
  - 5 volunteers (6%) with bili ≥ 1.5X ULN

- Renal toxicity in 6 volunteers terminated development
  - Acute renal insufficiency
  - 5 hospitalized/ 1 outpatient
  - 1 volunteer required prolonged dialysis

DB289 Study Treatment Protocol Mouse Diversity Panel



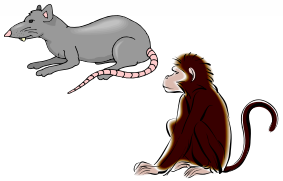
UC = Urine Collection in metabolism cages

- Endpoints
  - Daily body weight, liver weight, spleen weight, clinical chemistry, histopathology, novel kidney biomarkers, metabolomics in liver and kidney, kidney tissue drug concentration

Harrill et al. Tox Sci. 2012

Could the MDP Approach Detect the Kidney Toxicity Liability?

Preclinical testing

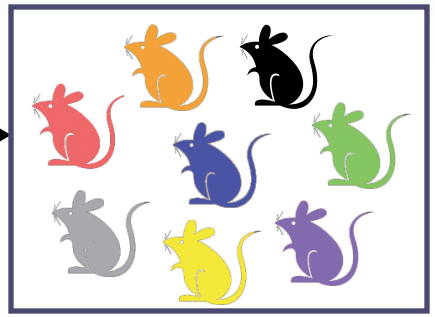


Predicted: efficacy, hepatotoxicity
Nor predicted: renal toxicity

Clinical Testing

Hepatotoxicity,
Renal insufficiency in expanded safety Phase I

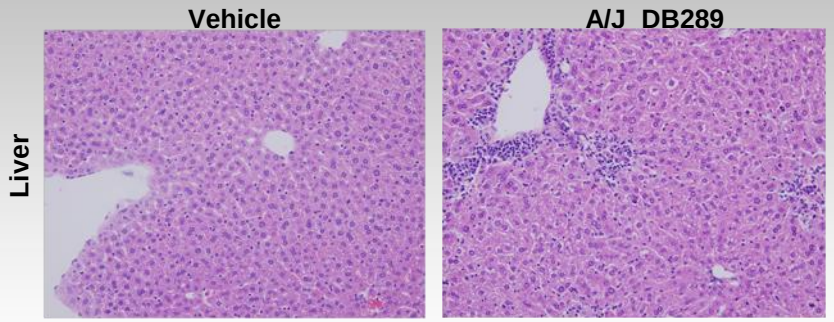
Mouse Diversity Panel



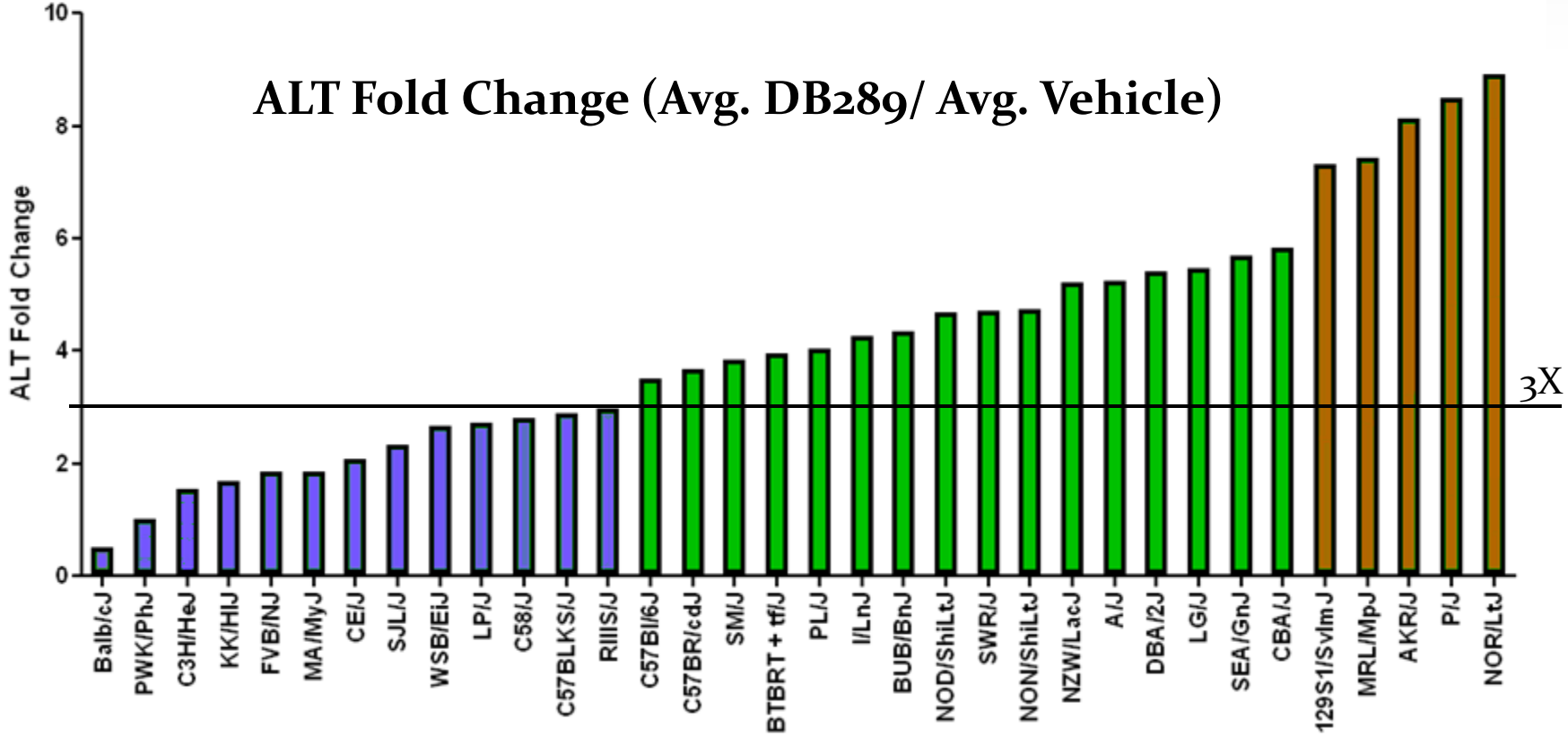
Hypothesis: Susceptible strains will detect renal toxicity liability, Mechanistic insight, biomarkers

Inform rational design of next-in-class

Provide biomarkers or sensitive strains to test

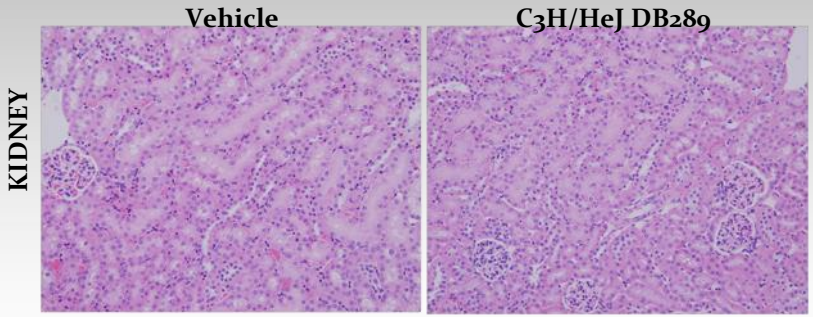


Liver Injury: ALT

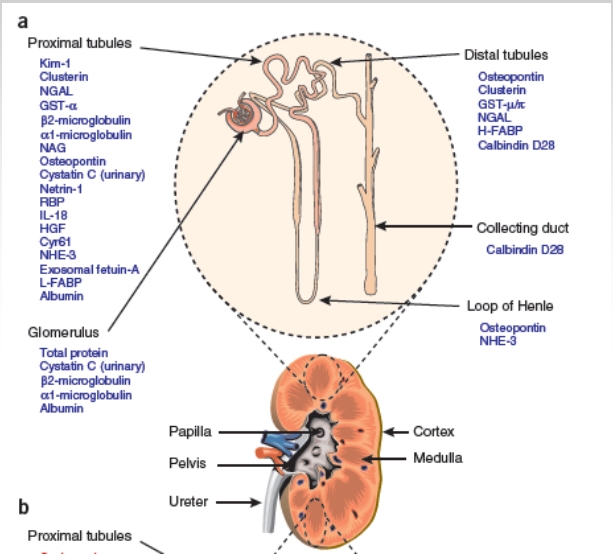


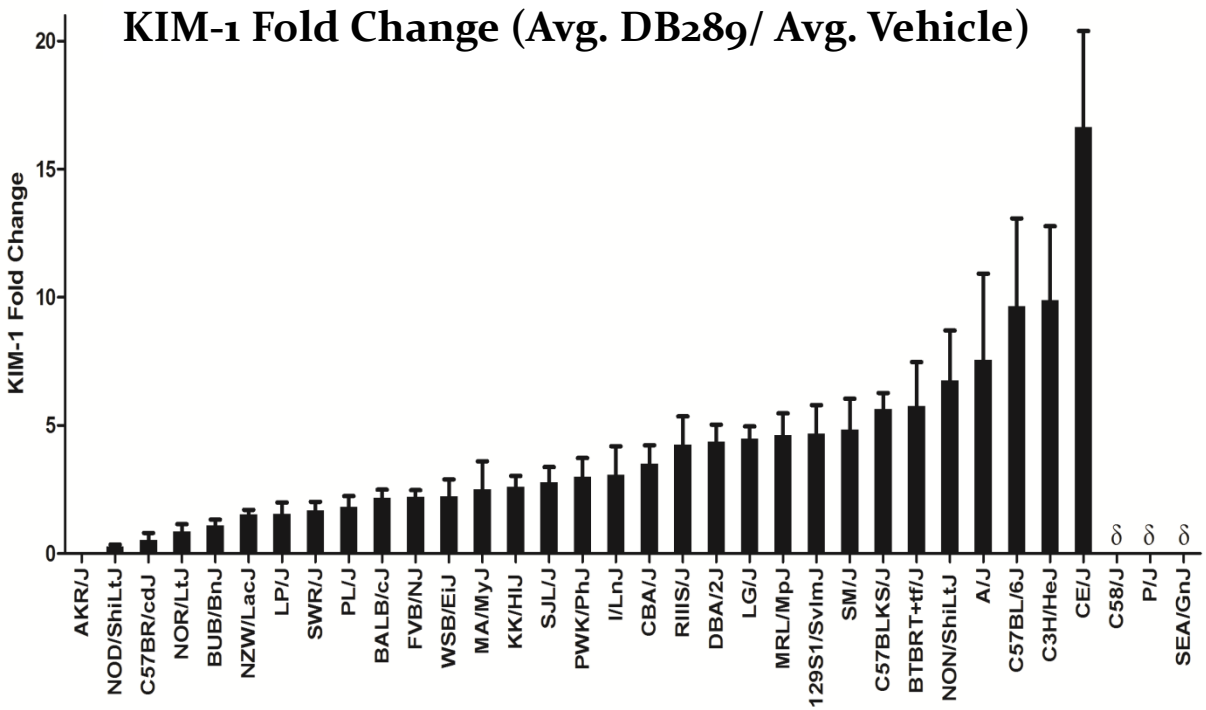
Harrill et al. Tox Sci. 2012

Kidney Injury: KIM-1



Histologic pathology was not observable in the majority of strains

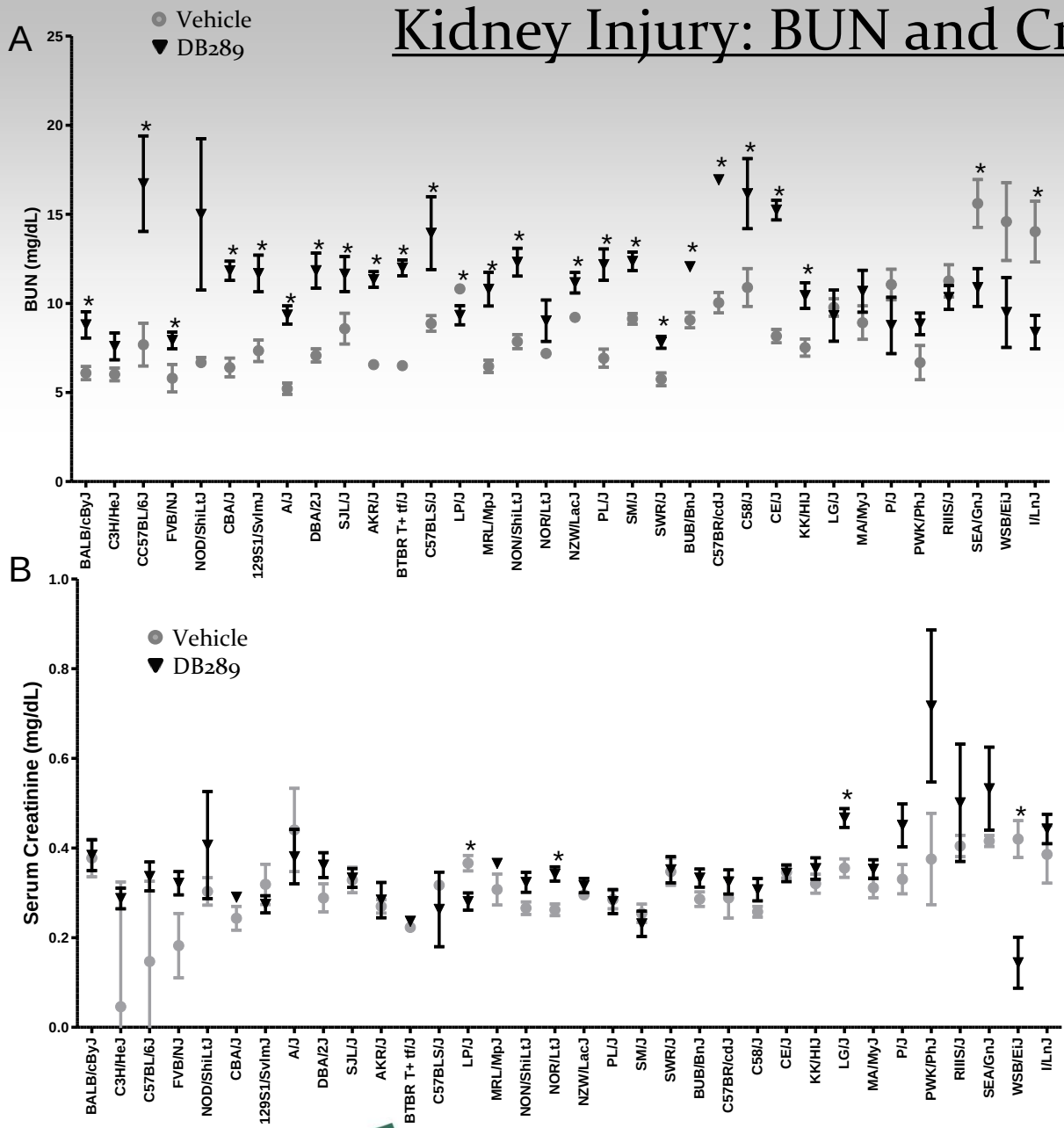




MDP approach enabled detection of clinical liver and kidney toxicity risk. Kidney toxicity risk would not have been detected in circulation without the use of sensitive biomarker KIM-1.

Harrill et al. Tox Sci. 2012

Kidney Injury: BUN and Creatinine



BUN slightly elevated for most strains

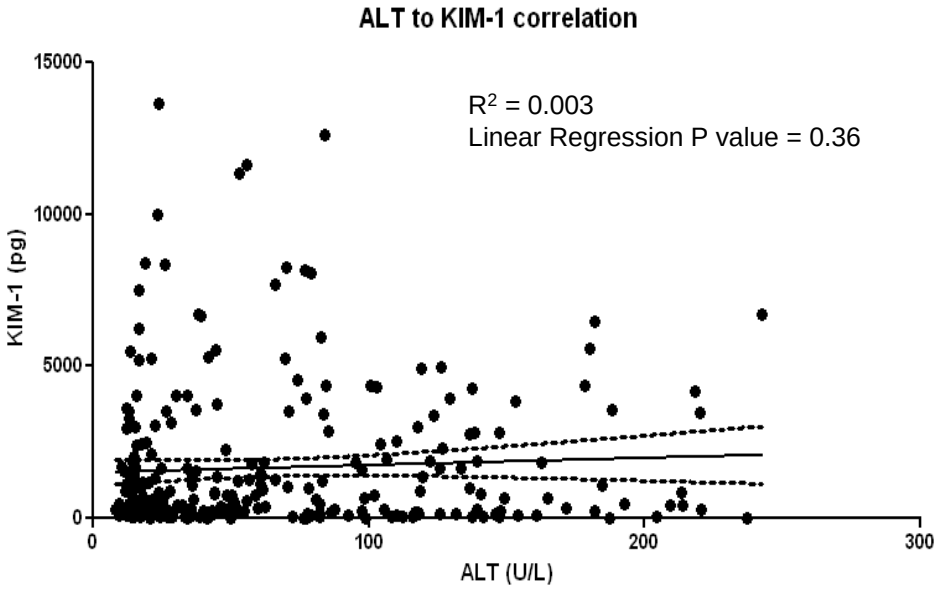
All values within normal range

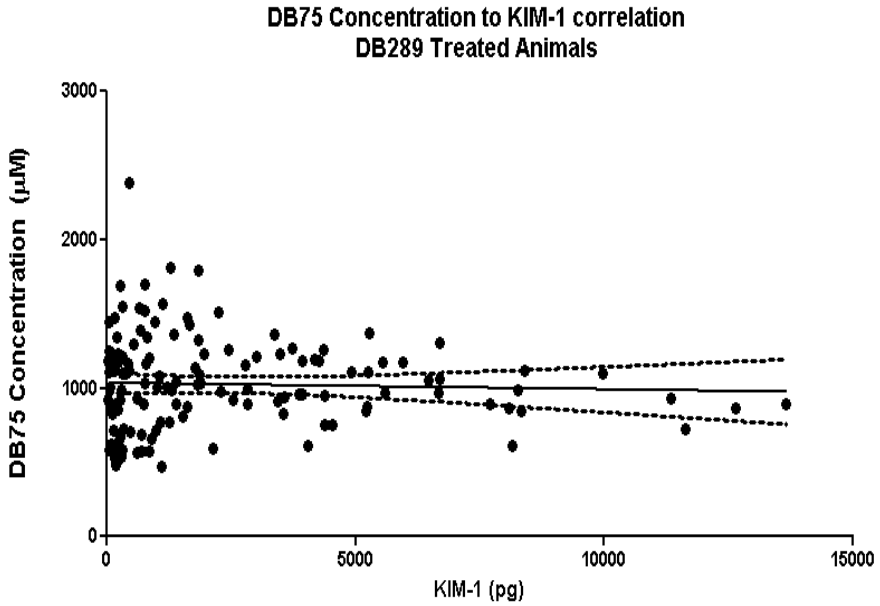
Creatinine slightly elevated for 2 strains, slightly decreased for 2 strains

All values within normal range

Harrill et al. Tox Sci. 2012

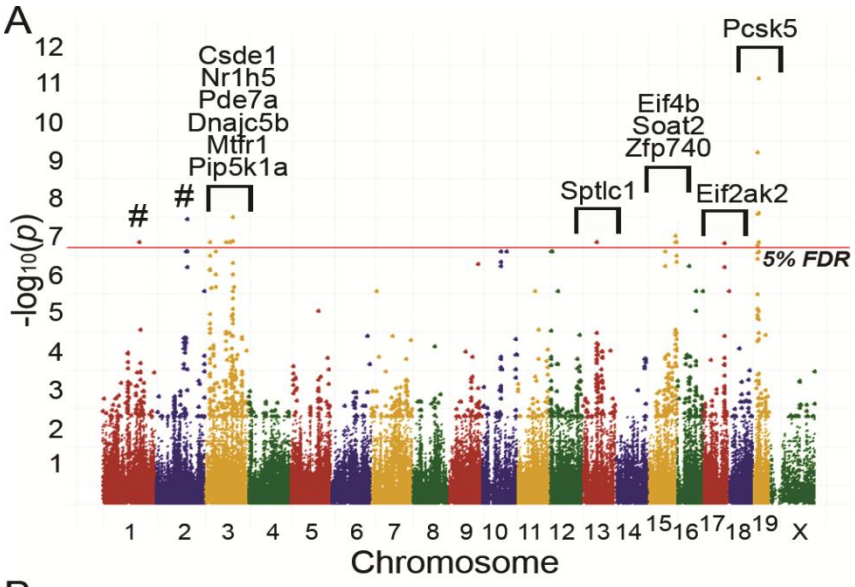
Correlation of Endpoints

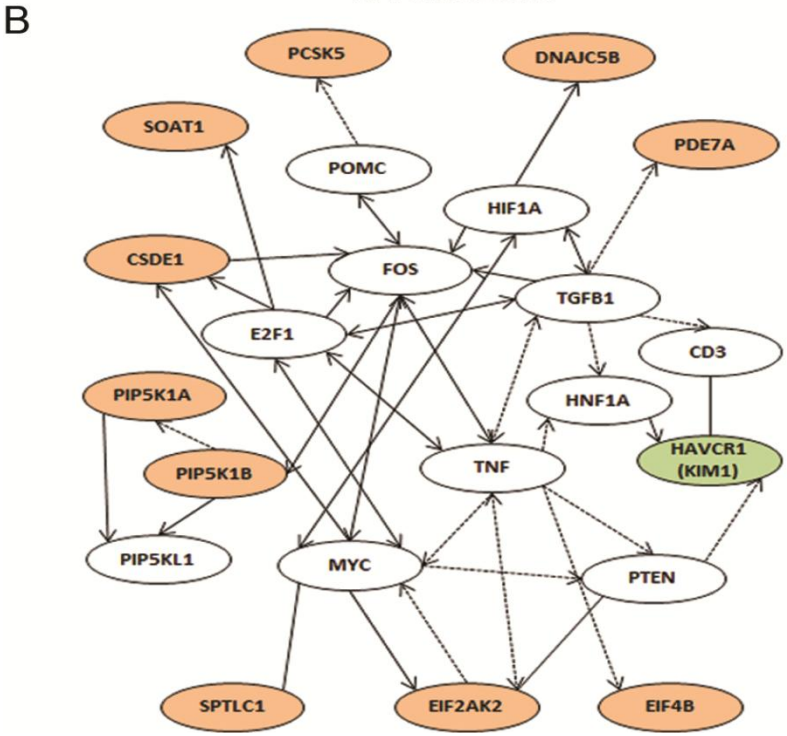




ALT and KIM-1 were not correlated with each other or with tissue drug concentration. Data indicated that distinct mechanisms of injury may affect the two tissues and that these were likely unrelated to strain differences in drug exposure/PK.

Harrill et al. Tox Sci. 2012





GWA identified gene variants that affect kidney susceptibility to DB289.

QTL genes were involved in cellular proliferation. KIM-1 levels directly reflect the rate of proliferation of renal proximal tubule cells and many of these genes regulate the function of KIM-1.

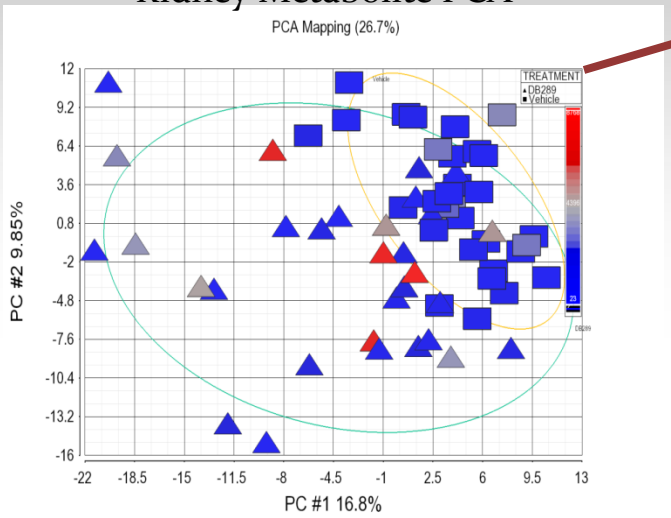
Metabolomics Analysis

Statistical Summary		
Welch's Two-Sample t-Test	**DB289 Vehicle**	
	Liver	**Kidney**
Total biochemicals $p \leq 0.05$	241	152
Biochemicals (↑|↓)	140|101	95|57
Correlation to Injury Markers	**ALT**	**KIM-1**
Total biochemicals $p \leq 0.05$	201	27

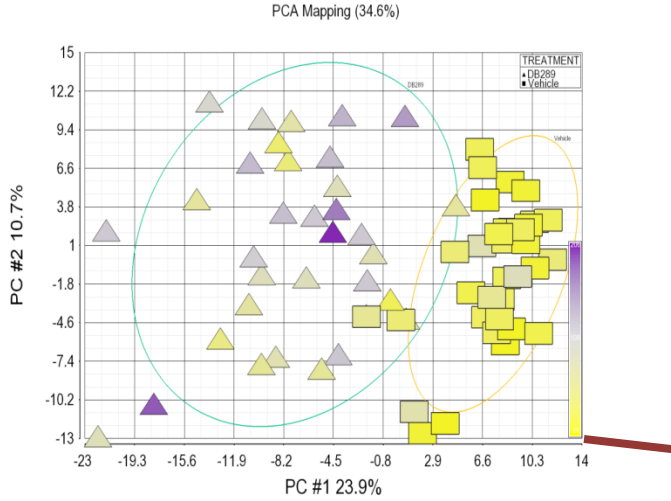
Harrill et al. Tox Sci. 2012

Differences in Organ Response: Kidney vs. Liver

Kidney Metabolite PCA



Liver Metabolite PCA



Same:

- Increased glutathione recycling
- Increased amino acid levels
- Increased sphingolipid turnerover (membrane turnover)

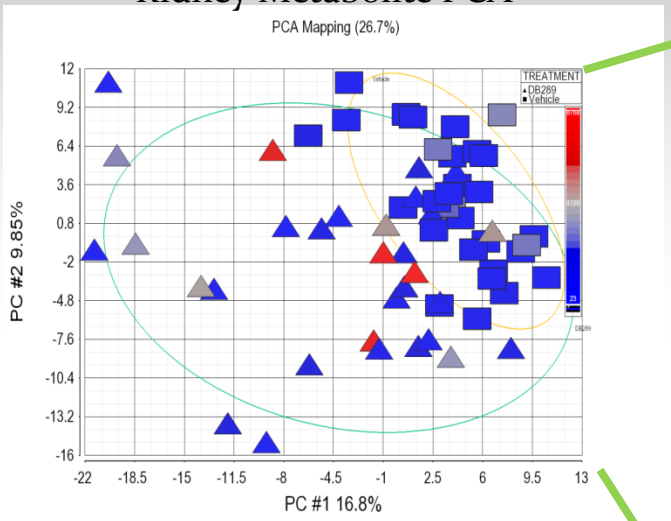
Different:

- Liver:
  - Reduced oxidation of fatty acids
  - Increased storage of fatty acids
  - Decreased synthesis of bile acids
- Kidney:
  - Few fatty acid changes
  - Increased long chain fatty acids
  - Elevation of bile acids, suggesting decreased renal elimination

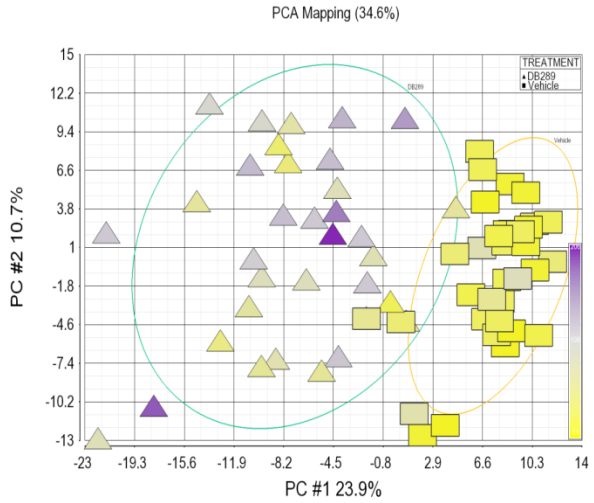
Unpublished Data

DB289 Metabolomics

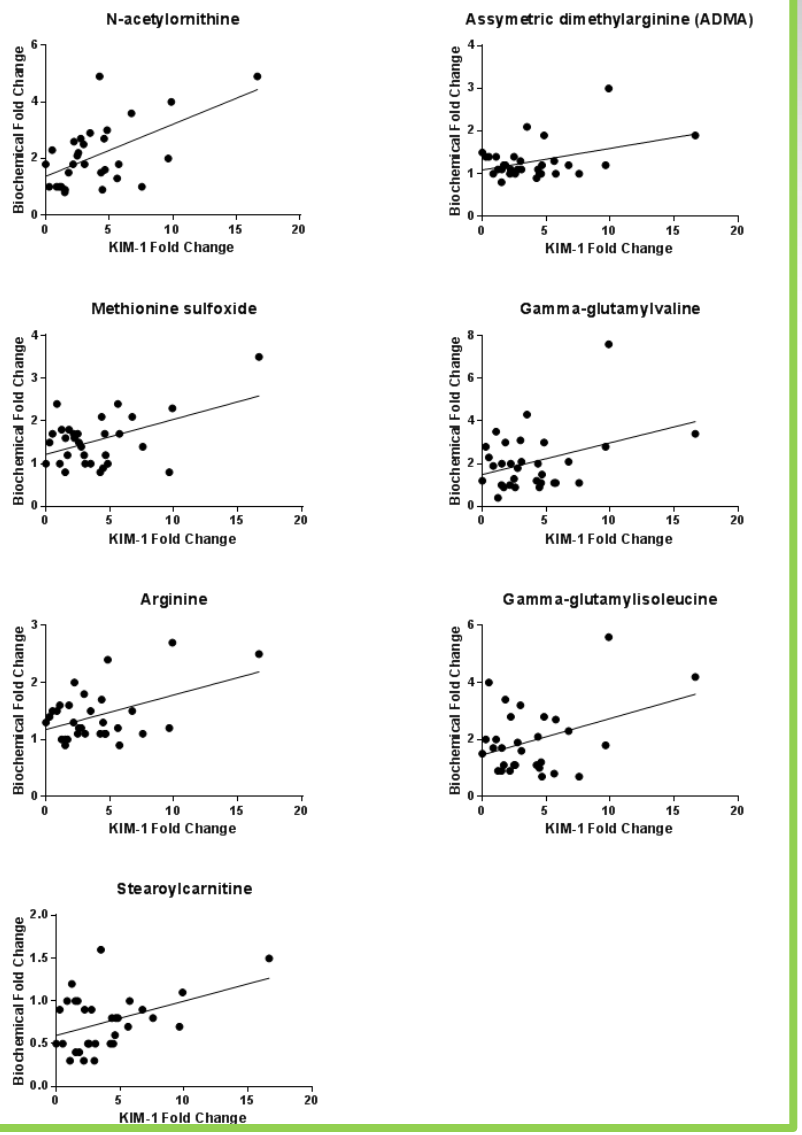
Kidney Metabolite PCA



Liver Metabolite PCA



Biomarkers of Kidney Response to DB289



Unpublished Data

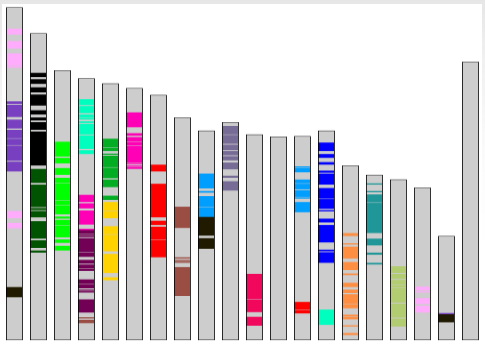
“Next-Gen” Mouse Population Models

- Rationally designed populations designed to maximize allelic diversity and randomize variation across the genome (avoid blind spots)
  - The Collaborative Cross
  - The Diversity Outbred

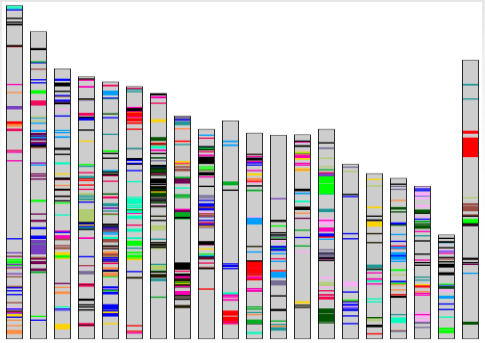
Comment on MDP vs. Collaborative Cross

Linkage Disequilibrium $R^2 = 0.1$

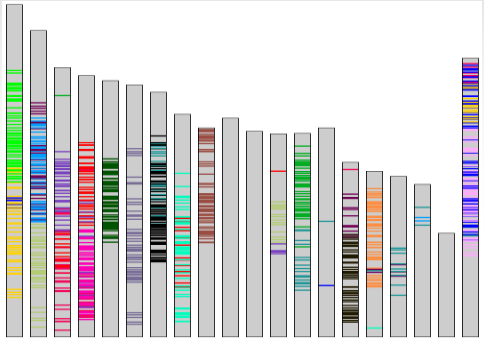
BXD (C57BL/6J x DBA/2J RI lines)



Mouse Diversity Panel



Collaborative Cross RI Lines



Data: Elissa Chesler, http://cgd.jax.org/datasets/ld/010.shtml

Many SNPs are in low level LD with other loci. For each panel, LD networks were anchored to the same 20 randomly selected genome locations and chromosomal distribution is shown (colors).

Result:
BXD (2 founders, many RI strains): LD is intra-chromosomal, but blocks are large (low precision)

MDP (34 strains): LD blocks small (high precision), but inter-chromosomal linkage pervasive (accuracy suffers)

CC: LD occurs in small, intra-chromosomal blocks not linked to other chromosomes (high accuracy, high precision)

Conclusions

- Genetically diverse mouse population-based approach was able to detect a genetic variant that predisposes to greater drug-induced liver injury in mice and humans due to acetaminophen

- Mouse Diversity Panel studies may offer improvements over classical approaches to detect clinical adverse drug reactions

- Genetically diverse mice offer insights into mechanisms of toxicity that occur within diverse populations

- Next-generation mouse models will improve mapping ability

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