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

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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

<table>
<thead>
<tr>
<th>Drug Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Depressants (SSRI’s)</td>
<td>38%</td>
</tr>
<tr>
<td>Asthma Drugs</td>
<td>40%</td>
</tr>
<tr>
<td>Diabetes Drugs</td>
<td>43%</td>
</tr>
<tr>
<td>Arthritis Drugs</td>
<td>50%</td>
</tr>
<tr>
<td>Alzheimer’s Drugs</td>
<td>70%</td>
</tr>
<tr>
<td>Cancer Drugs</td>
<td>75%</td>
</tr>
</tbody>
</table>

Percentage of the patient population for which a particular drug in a class is ineffective, on average.

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse Drug Reaction (ADR)</th>
<th>Risk Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir</td>
<td>Hypersensitivity</td>
<td>HLA-B*5701</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>Hepatotoxicity</td>
<td></td>
</tr>
<tr>
<td>Allopurinol</td>
<td>Cutaneous ADR</td>
<td>HLA-B*5801</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Stevens-Johnson Syndrome</td>
<td>HLA-B*1502</td>
</tr>
<tr>
<td>Augmentin</td>
<td>Hepatotoxicity</td>
<td>DRB1*1501</td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>Hepatotoxicity</td>
<td>DRB1*0701</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>Hepatotoxicity</td>
<td>HLA-A*3303</td>
</tr>
</tbody>
</table>

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
• 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

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

Photos by Stanton Short, Jackson Laboratory
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

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

Genetically Diverse Human 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

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)
  - Idiosyncrasy – DILI at apparent therapeutic doses
Acetaminophen Toxicity in Mouse and Human Populations

Mouse Phenotypic Responses

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

Human Phenotypic Responses

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

**Haplotype-Associated Mapping**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Peak</th>
<th>Genome position (Mb)</th>
<th>Genes in region</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 Hr ALT</td>
<td>1</td>
<td>Chr 2: 102.08-106.96</td>
<td>Trim44, E430002G05Rik, Slc1a2, Cd44, Pdhx, Apip, Ehf, BC016548, Elf5, Cat, Abtb2, Nat10, Gpiap1, Lmo2, 4931422A03Rik, Fbxo3, Cd598, Elf5, Cat, Abtb2, Nat10, Gpiap1, Lmo2, 4931422A03Rik, Fbxo3, Cd59b, Cd59a, A930018P22Rik, D430041D05Rik, Hipk3, Cstf3, Tcp11f1, AV216087, Qser1, Prpg4, Ccdc73, Ga17, Wt1, 0610012H03Rik, Rcn1, Pax6, Elp4, Immp1l, Zcsl3, 4732421G10Rik, Mpped2, 2700007P21Rik, Fshb</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Chr 3: 126.439-26.844</td>
<td>Ank2</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Chr 4: 141.531-43.578</td>
<td>Prdm2, Pdpn, Lrrc38, Pramel1, 4732496O08Rik, Oog4, BC080695, Pramel5, Pramel4, Oog3</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Chr 6: 123.795-24.766</td>
<td>V2rb1, Cd163, Pex5, Clstn3, C1rl, C1r, Oact5, Emg1, Phb2, Ptn6, Grcc10, Atn1</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Chr 13: 36.862-37.022</td>
<td>Ly86</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>Chr 17: 5.598-5.655</td>
<td>Zdhhc14</td>
</tr>
<tr>
<td>24 Hr ALT</td>
<td>7</td>
<td>Chr 1: 182.602-82.719</td>
<td>Capn8</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Chr 2: 127.489-27.580</td>
<td>Bub1, 1500011K16Rik</td>
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<tr>
<td>9</td>
<td>9</td>
<td>Chr 4: 124.084-24.395</td>
<td>Utp11f, Fhl3, St3a3, Inpp5b, Mtf1, Yrdc, Gm50, Epha10, Cdca8, 9930104L06Rik</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>Chr 5: 97.392-97.681</td>
<td>Prdm8, Fgf5, 1700007G11Rik</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>Chr 7: 86.492-86.594</td>
<td>No known genes</td>
</tr>
</tbody>
</table>

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 -- *CD*$_{44}$

**UNC + Purdue Pharma Study Population**

- 121 APAP subjects
- 50 mg/kg (2 tablets/6hr)

*CD*$_{44}$ Polymorphism Allele Frequency in 275 Caucasian Acute Liver Failure Subjects

- Reference Population (Caucasian)
- Non-APAP Acute Cases (n=114)
- Acute Intentional APAP (n=81)
- Chronic Unintentional APAP (n=80)

**Graph:**
- Mean Serum ALT (U/L) vs. Treatment Day
- C/C, N=100
- C/T, N=20
- T/T, N=1

**Results:**
- *P = 0.014 by Chi-squared test

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)


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
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

-14  -1  1  2  3  4  5  6  7  8  9  10  11

-18 HR  Dose 1  Dose 2  Dose 3  Dose 4  Dose 5  Dose 6  Dose 7  Dose 8  Dose 9  Dose 10  Necropsy

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

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

ALT Fold Change (Avg. DB289/ Avg. Vehicle)

Kidney Injury: KIM-1

Histologic pathology was not observable in the majority of strains.

KIM-1 Fold Change (Avg. DB289/ Avg. Vehicle)

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.
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
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.

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.

**Statistical Summary**

<table>
<thead>
<tr>
<th>Welch's Two-Sample t-Test</th>
<th>DB289 Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>241</td>
</tr>
<tr>
<td>Kidney</td>
<td>152</td>
</tr>
<tr>
<td>Total biochemicals</td>
<td></td>
</tr>
<tr>
<td>$p \leq 0.05$</td>
<td>140</td>
</tr>
<tr>
<td>Correlation to Injury Markers</td>
<td>ALT</td>
</tr>
<tr>
<td>Total biochemicals</td>
<td>201</td>
</tr>
<tr>
<td>$p \leq 0.05$</td>
<td>27</td>
</tr>
</tbody>
</table>

Metabolomics Analysis

**Differences in Organ Response: Kidney vs. Liver**

**Same:**
- Increased glutathione recycling
- Increased amino acid levels
- Increased sphingolipid turnover (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

Biomarkers of Kidney Response to DB289

Kidney Metabolite PCA

Liver Metabolite PCA

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 $R^2 = 0.1$

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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