Mouse Populations Enable Translational Pharmacogenomic Approaches for Understanding and Predicting Adverse Drug Events

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Disclaimer

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The pharmaceutical industry is facing a **productivity crisis**:

Rate of FDA approvals of new drugs fell by 50% in the past decade
- toxicity a greater attrition factor than in the past

**Drug safety becoming a major bottleneck in regulatory approval as the science of drug safety lags behind that of drug efficacy**
Even worse – Drugs that appear safe initially may cause severe toxicities in sensitive patients

- Toxicities of (selected) manufactured drugs occurring in the recent modern regulatory era:
  - 2000: Troglitazone (diabetes): idiosyncratic hepatotoxicity
  - 2004: Vioxx (NSAID): myocardial infarction
  - 2006: Exanta (anticoagulant): idiosyncratic hepatotoxicity
  - 2008: Lumiracoxib (COX-2 inhibitor): idiosyncratic hepatotoxicity
  - 2010: Sibutramine/Meridia (anorexiant): stroke, cardiovascular events
1. Modernize toxicology to enhance product safety
2. Stimulate innovation in clinical evaluations and personalized medicine
3. Support new approaches to improve product manufacturing and quality
4. Ensure FDA readiness to evaluate emerging technologies
5. Harness diverse data through information sciences to improve health outcomes
6. Implement a prevention-focused food safety system to protect public health
7. Facilitate development of medical countermeasures
8. Strengthen social and behavioral science to help make informed decisions about regulated product

Attrition During Drug Development is Costly

Standard animals: How much like humans are they?

Even Humans Aren’t a Good Model for Humans!

<table>
<thead>
<tr>
<th></th>
<th>Percentage</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Anti-Depressants (SSRI’s)</td>
<td>38%</td>
<td><img src="image1.png" alt="Human Figures" /></td>
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<tr>
<td>Asthma Drugs</td>
<td>40%</td>
<td><img src="image2.png" alt="Human Figures" /></td>
</tr>
<tr>
<td>Diabetes Drugs</td>
<td>43%</td>
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<tr>
<td>Arthritis Drugs</td>
<td>50%</td>
<td><img src="image4.png" alt="Human Figures" /></td>
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<tr>
<td>Alzheimer’s Drugs</td>
<td>70%</td>
<td><img src="image5.png" alt="Human Figures" /></td>
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<tr>
<td>Cancer Drugs</td>
<td>75%</td>
<td><img src="image6.png" alt="Human Figures" /></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."
Using Genetically Diverse Mouse Populations to Identify Safety Concerns and Genes Associated with Toxicity

Genetically Diverse Mouse Population

Genetically defined rodent populations may provide ability to:
1. Better predict safety
2. Identify genetic factors that predict an individual patient’s risk (or benefit), thereby:
   - Allowing otherwise efficacious drugs to remain on the market
   - Providing insight into mechanisms that guide design of next-in-class compounds

Translate results

Genetically Diverse Human Population

Additional benefits:
• Ethical concerns might preclude treating humans with a compound known to be toxic, but for which the mechanisms remain to be elucidated
• Some adverse reactions are so rare that it is a major challenge to identify and catalog samples from enough patients to sufficiently power GWAS
Genetically Diverse Mouse Populations – Implementation #1:

Identification of Genetic Risk Factors for Drug Toxicity
Personalize Medicine for Each Patient

**Molecular Taxonomy of Disease:** Classifying the patient’s disease by molecular or genetic signature instead of symptoms
Acetaminophen Toxicity in Mouse and Human Populations

Acetaminophen-induced liver injury

Liver toxicity: Humans
~57 mg/kg

Liver toxicity: Mouse population
300 mg/kg

RESULT: Enrichment of CD44 variant in ALF cases due to chronic acetaminophen use.

Pharmacogenetics of EGCG DILI

- Epigallocatechin gallate (EGCG) is a minor component of green tea with antioxidant properties

- Concentrated green tea extract herbal supplement use has led to rare cases of severe DILI with no obvious link to dose

- **Key collaborators:** Gary Churchill and Dan Gatti (Jackson Laboratory); David Threadgill (NC State), Yi Xang, Qiang Shi (NCTR), Paul Watkins, Tom Urban (DILIN), Rachel Church (former postdoc)
A Sensitive Subpopulation Identified Using Diversity Outbred Mice

Church et al. Tox Sci. 2014
### Translation to Human Patients

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>SNP (Array)</th>
<th>Gene Name</th>
<th>Chromosome</th>
<th>Position</th>
<th>P value</th>
<th>Risk/Protective allele</th>
<th>Effect</th>
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<tbody>
<tr>
<td>RNF207</td>
<td>exm8990</td>
<td>ring finger protein 207</td>
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<td>627841</td>
<td>0.00478</td>
<td>G/A</td>
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<td>ring finger protein 207</td>
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<td>627937</td>
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<td>G/C</td>
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<tr>
<td>PER3</td>
<td>exm10762</td>
<td>period circadian clock 3</td>
<td>1</td>
<td>788723</td>
<td>0.00493</td>
<td>T/C</td>
<td></td>
</tr>
<tr>
<td>MFN2</td>
<td>exm15928</td>
<td>mitofusin 2</td>
<td>1</td>
<td>120696</td>
<td>0.0067</td>
<td>A/G</td>
<td>Missense (I/V)</td>
</tr>
<tr>
<td>GPR153</td>
<td>exm9066</td>
<td>G protein-coupled receptor 153</td>
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<td>631056</td>
<td>0.01670</td>
<td>C/G</td>
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<tr>
<td>VPS13D</td>
<td>exm16480</td>
<td>vacuolar protein sorting 13 homolog D (S. cerevisiae)</td>
<td>1</td>
<td>123434</td>
<td>0.04306</td>
<td>A/T</td>
<td></td>
</tr>
</tbody>
</table>

- In this case, whole genome SNP genotyping had been performed on patient samples for another study.

- Mitofusin 2: Involved in mitochondrial fusion and autophagy.

- Previous study confirmed EGCG inhibits respiratory complexes in stressed mitochondria  
  Weng et al. Biochem Biophys Res Commun 2014
Genetically Diverse Mouse Populations – Implementation #2:

Improve Prediction of Human Toxicity Risk & Mechanistic Analysis
PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity

Key Collaborators: Karissa Adkins & Hong Wu (Pfizer); Merrie Mosedale (former postdoc fellow)

• PF-04287881 -
  – Ketolide antibiotic under development at Pfizer
  – Like other cationic amphiphilic antibiotics, there were indications of the presence of phospholipidosis and liver injury in rodents and dog
    • Other examples: azithromycin and erythromycin
    • Well tolerated at 30X the anticipated clinical exposure

  – Development discontinued following mildly increased LFTs in Phase 1 trial
    • Inter-individual variation observed

Mosedale et al. TAAP 2014.
PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity

- **Goal 1**: Identify sensitive and resistant strains of mice to 881 hepatotoxicity
- **Goal 2**: Characterize the injury
- **Goal 3**: Elucidate molecular pathways that distinguish responders from nonresponders
PF-04287881, Novel antibiotic that failed Phase I Clinical Trials due to Hepatotoxicity

- Dose range finding study in CD-1 mice
- 600 mg/kg (i.g) or vehicle for 7 days
- 34 inbred strains (classical MDP)

Mosedale et al. TAAP 2014.
RESULT: PF-04287881 elicited liver toxicity in a majority of strains tested. Identified responder and nonresponder strains.

Mosedale et al. TAAP 2014.
RESULT: ALT did not correlate with plasma drug concentration or AUC.
COMMON MISCONCEPTION: You have to do every mouse population-based experiment in 300-400 animals.

FACT: Not necessarily the case. Once you have determined sensitive genetic backgrounds, you can focus your follow up studies in selected strains.

This is also true for the DO mice once the genetic basis for the phenotype has been established.

RESULT: Degree of KC vacuolation is correlated with ALT.

Mosedale et al. TAAP 2014.
Characterization of histologic vacuoles as phospholipidosis

**RESULT:** Of 4 strains tested

The two strain with KC vacuolation (H&E) had characteristic phospholipidosis (EM)

Present in liver, lung, and spleen

Mosedale et al. TAAP 2014.
PF-04287881 Caused Single Cell Necrosis in Sensitive Strains that was Correlated with ALT

**RESULT:** miR-122 release into plasma confirmed liver origin of ALT.

Transcripts for which expression changes were significant by responder status and by treatment group were highly associated with protein ubiquitination and lysosomal function.

Table 1

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene title</th>
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<tbody>
<tr>
<td><strong>Decreased</strong></td>
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<tr>
<td>Psmb5</td>
<td>proteasome subunit, beta type, 5</td>
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<tr>
<td>Psmc5</td>
<td>proteasome 26S subunit, ATPase, 5</td>
</tr>
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<td>Psmc3</td>
<td>proteasome 26S subunit, ATPase, 3</td>
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<td>Psmc4</td>
<td>proteasome 26S subunit, ATPase, 4</td>
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<tr>
<td>Psma6</td>
<td>proteasome subunit, alpha type, 6</td>
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<tr>
<td>Ube2g2</td>
<td>ubiquitin-conjugating enzyme E2G 2</td>
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<tr>
<td>Psmc6</td>
<td>proteasome 26S subunit, ATPase, 6</td>
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<tr>
<td>Hspa9</td>
<td>heat shock 70kDa protein 9</td>
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<tr>
<td>Usp48</td>
<td>ubiquitin specific peptidase 48</td>
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<tr>
<td>Psmd6</td>
<td>proteasome 26S subunit, non-ATPase, 6</td>
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<td>proteasome subunit, alpha type, 5</td>
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<td>Psmd4</td>
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<tr>
<td>Hspa1a/Hspa1b</td>
<td>heat shock 70kDa protein 1A</td>
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<tr>
<td>Hspb6</td>
<td>heat shock protein, alpha-crystallin-related, B6</td>
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<td>Psmd7</td>
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<td><strong>Increased</strong></td>
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<tr>
<td>Dnaje16</td>
<td>DnaJ (Hsp40) homolog, subfamily C, member 16</td>
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<tr>
<td>Ube4b</td>
<td>ubiquitination factor E4B</td>
</tr>
<tr>
<td>B2m</td>
<td>beta-2-microglobulin</td>
</tr>
</tbody>
</table>

Mosedale et al. TAAP 2014.
DB289 - Test case for improving prediction of human-relevant adverse events

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

Kidney toxicity risk would not have been detected in circulation without the use of sensitive biomarker KIM-1.

Isoniazid Hepatotoxicity

- **MILD**: 10-20% patients; ATs < 100 U/L, focal hepatocellular damage on biopsy (Nolan et al. *JAMA* 1999); mild AT elevations resolve despite continued therapy
  - In some cases, physicians opt to “treat through” AT elevations

- **SEVERE**: 1-2% patients; hepatitis and liver failure, combination therapy for active TB infection increases incidence (i.e. rifampin)
  - Most common cause of acute liver failure due to DILI in some countries (ex. India)
### Isoniazid *in vivo* Experimental Design

**Drug Exposure (3 Days):**

-14 0 1 2 3

Study Day: Fast Fast

- **Isoniazid 100 mg/kg *i.g.* daily**

- **Necropsy**

**Endpoints of Interest:**

1. **Histopathology, Adipophilin** – Hamner/EPL, Pfizer, NIH (NIDDK)
2. **Cytokine profiles** – J&J (Ameesha Batheja, Monica Singer)
3. **miRNA (miR-122) Expression** – J&J (Ameesha Batheja, Monica Singer)
4. **Transcriptomics** – Hamner (Affymetrix arrays donated by Lilly, AstraZeneca, and Pfizer)
5. **Metabolomics** – RTI (Susan Sumner)
6. **Genomics (GWAS):** Hamner, Pfizer
7. **Cholesterol/Triglycerides:** Hamner, Pfizer

**Collaborations with HESI Genomics Technical Committee**
RESULT: About half of strains tested experienced a positive steatotic response.
RESULT: Liver cholesterol and Serum LDL levels correlated with the extend of liver steatosis.

RESULT: GWAS identified a few significant peaks, one contained plin2, the gene encoding adipophilin. Adipophilin itself is often used as an IHC marker of steatosis.
RESULT: Transcripts changed by treatment and differentially expressed in liver in sensitive strains are largely associated with lipid packaging (i.e. lamp1).
All Samples: Metabolites important for the separation of pathology groups (0-1 vs 2-5)

RESULT: Liver metabolites that distinguished responders enriched for metabolites associated with betaine homocysteine methyltransferase pathway

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>pValue</th>
<th>Incr/Decr</th>
<th>Metabolite</th>
<th>pValue</th>
<th>Incr/Decr</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Aminoadipate</td>
<td>&lt;0.050</td>
<td>Incr</td>
<td>Lactate</td>
<td>&lt;0.100</td>
<td>Decr</td>
</tr>
<tr>
<td>3-Hydroxybutyrate</td>
<td>&lt;0.100</td>
<td>Decr</td>
<td>Leucine</td>
<td>&lt;0.100</td>
<td>Decr</td>
</tr>
<tr>
<td>Acetate</td>
<td>&lt;0.100</td>
<td>Decr</td>
<td>Lipoproteins</td>
<td>&lt;0.100</td>
<td>Incr</td>
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<tr>
<td>Adenine</td>
<td>&lt;0.100</td>
<td>Decr</td>
<td>O-Phosphocholine/O-Acetylcholine/sn-Glycerophosphocholine/Carnitine</td>
<td>&lt;0.005</td>
<td>Decr</td>
</tr>
<tr>
<td>Alanine</td>
<td>&lt;0.050</td>
<td>Decr</td>
<td>Ornithine</td>
<td>&lt;0.100</td>
<td>Decr</td>
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<td>Ascorbate</td>
<td>&lt;0.010</td>
<td>Decr</td>
<td>Phenylalanine</td>
<td>&lt;0.005</td>
<td>Decr</td>
</tr>
<tr>
<td>Betaine/ TMAO</td>
<td>&lt;0.050</td>
<td>Decr</td>
<td>Serine</td>
<td>&lt;0.100</td>
<td>Decr</td>
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<tr>
<td>Cadaverine</td>
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<td>Decr</td>
<td>Taurine</td>
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<td>Tyrosine</td>
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<td>Incr</td>
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<td>Incr</td>
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<td>Incr</td>
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<td>Decr</td>
<td>Unsaturated lipids</td>
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<td>Isoleucine</td>
<td>&lt;0.050</td>
<td>Decr</td>
<td></td>
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</table>
Effect of Isoniazid Treatment on Lipid Export in Genetically Sensitive Mice

RESULT: All omics data combined together into one unifying hypothesis regarding the mechanism of INH-induced steatosis.

Key:
- Increased
- Decreased

Church et al. Tox Sci. 2014
Next Steps for the Harrill Lab

• Investigation of the DO mice as a tool to detect and predict DILI (Collaboration with Jax and FDA)
  – Screen drugs known to cause idiosyncratic DILI
  – Screen drugs that failed in clinical development
  – GWAS on selected candidates

• Mechanistic analysis of the DILI reaction for various drugs (now that we have a model – we can study it!)

Visit Lascelles Lyn-cook’s poster to see our preliminary data on using the DO for drug safety assessment.
Conclusions

• Genetically diverse mouse population-based approach was able to detect a genetic variant associated with DILI sensitivity for:
  – Dose dependent hepatotoxic acetaminophen
  – Idiosyncratic hepatotoxin EGCG

• Genetically diverse mouse populations may offer improved ability over classical models to detect human toxicity risk:
  – 881 – liver injury
  – DB289 – renal injury

• Genetically diverse mice offer insights into mechanisms of toxicity that occur within sensitive individuals
  – Isoniazid – hepatic steatosis
  – 881 - phospholipidosis
In the world of drug safety science,

THE ROAD TO SUCCESS IS ALWAYS UNDER CONSTRUCTION

-Larry Wall