Identifying G X E Interactions and Thresholds for Toxicity in Diversity Outbred Mice

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Special Volunteer NTP

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Outline

• **Role and relevance** – inbred homozygous strains vs. Diversity Outbred & CC RIL mice

• **Proof of concept**: Benzene, an environmental genotoxicant and leukemogen

• **Benzene metabolites** — chromosomal damage associated with the development of leukemia

• **Benchmark Concentration Models** – point of departure

• **Mapping resistance and/or susceptibility QTLs**

• **Future directions**
Genetic and Epigenetic Basis for Susceptibility

Link individual exposure to biology and to disease
(Adapted from National Research Council, 1987)
Addressing Human Variability in Next-Generation Human Health Risk Assessments of Environmental Chemicals

Lauren Zeise, Frederic Y. Bois, Weihsureh A. Chiu, Dale Hattis, Ivan Rusyn, and Kathryn Z. Guyton

Types of biological variability

- Heredity (genetic and epigenetic)
- Sex, lifestage, and aging
- Existing health conditions
- Coexposures (sources outside decision context)
- Food/nutrition
- Psychosocial stressors

Susceptibility indicators

- Exposure parameters
- Background and coexposure doses
- PK parameters
- Endogenous concentrations
- PD parameters
- Baseline biomarker values
- Systems parameters
- Outcome latency, likelihood, and severity

Source-to-outcome continuum

- Source/media concentrations
  - Multiple sources leading to chemicals in multiple media
- Exposure
  - External doses
    - Multiple chemicals via multiple routes
- Pharmacokinetics
  - Internal concentrations
    - Multiple chemicals (including metabolites) at multiple target sites
- Pharmacodynamics
  - Rinoigical response measurements
    - Multiple biological responses in multiple tissues/biological media
- Systems dynamics
  - Physiological/health status

Modifying how changes in source/media concentrations are propagated to changes in outcome.

For fixed source/media concentrations, modifying the background or baseline conditions.
Brief Background - Toxicogenetics

- Integration of genetics with toxicology
- Inter-individual variation in genetics and environmental exposure (uncontrolled variables)
- Hazard identification and characterization – false negative and false positive error rates?
- Corroborate genetic epidemiology and/or provide an effective tool for safety assessment
Benzene 28 Day Inhalation Exposure: Proof of Concept

- Diversity outbred (J:DO) male mice: 7 & 8th randomly outbred generations; selected from 175 breeding pairs
- Randomly assigned to exposure groups by weight
- Dose levels: 0, 1, 10, 100 ppm benzene, 28 days, 6 hr/day
- 75 male mice per exposure group, 300 mice/study
- 2 independent cohorts to assess reproducibility (600 mice total)
- Endpoints for hematotoxicity and genetic damage
  - % reticulocytes and micronucleated reticulocytes in peripheral blood and bone marrow
  - Mouse Universal Genotyping Array (9K SNPs)
  - Linkage mapping analysis (DO QTLRel)

Benzene metabolism and toxicity


- Human & rodent hematotoxicant and carcinogen
- Metabolized in liver and bone marrow
- Human & rodent ADME (toxicokinetics) are comparable
The results....
Benchmark Concentration Models
DO vs. B6C3F1 (Farris et al. 1996)
<table>
<thead>
<tr>
<th>Quartile</th>
<th>BMC</th>
<th>BMCL_{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>All quartiles</td>
<td>0.367</td>
<td>0.200</td>
</tr>
<tr>
<td>Q1</td>
<td>0.550</td>
<td>0.205</td>
</tr>
<tr>
<td>Q2+Q3</td>
<td>0.315</td>
<td>0.117</td>
</tr>
<tr>
<td>Q2+Q3+Q4</td>
<td>0.275</td>
<td>0.130</td>
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<tr>
<td>Q4</td>
<td>0.257</td>
<td>&lt;0.001</td>
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</table>

BMCL_{10} = 0.205 ppm

0.205/0.001 ppm is ≈200X

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Benchmark Concentration Model (BMC)

EPA Risk Assessment 1988 (Updated & revised 2003)

BMCL* = 8.2 mg/m³ (2.6 ppm); UF = 300; MF = 1

RfC** = 3 x 10⁻² mg/m³ (9.4 ppb)

*Decreased lymphocyte count in 44 individuals (Rothman et al., Am. J. Ind. Med. 29:236, 1996). No metric provide (i.e. 1SD or 10% above the control mean).

Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio)

DO mouse population linkage mapping

- Genetically heterogeneous reference population
- Derived from a set of 8 inbred founder strains (from the Collaborative Cross)
- 45 million segregating SNPs, indels, & CNV
- Balanced founder allele (1/8) frequencies averaging 12%
- Combination of high genetic diversity, low MAF, and fine recombination block structure make the DO mice ideal for genetic mapping

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Name / description</th>
<th>Chr Location (GRChr)</th>
<th>Size</th>
<th>Alert/Location</th>
<th>Identifier</th>
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<tbody>
<tr>
<td>Nkain2</td>
<td>Na+/K+ transporting ATPase interacting 2</td>
<td>10:31689319-32889915 (→)</td>
<td>1200596</td>
<td>Structural variant* Ins 31838075-31838077 del 32310195-32319195</td>
<td>MGI:1923447</td>
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<tr>
<td>Trdn</td>
<td>Triadin</td>
<td>10:33083483-33476709 (+)</td>
<td>393226</td>
<td>Nonsynonymous SNP* rs33783582 rs33784331 Structural variant del 33269490-33269848</td>
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<tr>
<td>Clvs2</td>
<td>Clavesin 2</td>
<td>10:33512286-33624769 (→)</td>
<td>112483</td>
<td>Structural variant del 33578793-33578899</td>
<td>MGI:2443223</td>
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<tr>
<td>Gm4794</td>
<td>Predicted gene 4794</td>
<td>10:33766424-337821</td>
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<tr>
<td>Sult3a1</td>
<td>Sulfotransferase family 3A, member 1</td>
<td>10:33863935-338794</td>
<td>15540</td>
<td>Structural variant gain 112K bp 33876195</td>
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<tr>
<td>Rsph4a</td>
<td>Radial spoke head 4 homolog A</td>
<td>10:33905485-33915883 (+)</td>
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<td>Zufsp</td>
<td>Zinc finger with UFM1 specific peptidase domain</td>
<td>10:33926936-33951212 (→)</td>
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<td>Nonsynonymous SNP* rs48254962</td>
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</table>
Expression QTL in DO mice (Liver)

A) Sult3a1

B) Gm4794

C) Founder Effect

D) LOD

Chr 10 (Mb)
Allele specific expression is the rule
Human copy number & 5mCpG variation

- **SULT1A1 phenol sulfotransferase**
  - CNV range 1-6 copies*

**Caucasian**: 5% - single copy; 69% - 2 copies; 26% -3 or more copies (**362 individuals**)

**African-Americans**: 0% - single copy; 38% - 2 copies; 62% - 3 or more copies (**99 individuals**)

*Hebbring et al. Human Molecular Genetics 16, 463, 2007
Gaedigk et al. Pharmacogenomics 13, 91, 2012*
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Name / description</th>
<th>Chr location (GRCm38)</th>
<th>Size</th>
<th>Alert</th>
<th>Identifier</th>
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<tr>
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<td>Predicted gene 14066</td>
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<td></td>
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<td>MGI:2444678</td>
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<tr>
<td>Tasp1</td>
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<td>233325</td>
<td>Structural Variant*</td>
<td>MGI:1923062</td>
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<tr>
<td>Esf1</td>
<td>ESF1, nucleolar pre-rRNA processing protein</td>
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<tr>
<td>Macrod2</td>
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<td>Kif16b</td>
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Summary & Conclusions

• DO mice show significant population variance
• Significant linkage mapping power
• Reproducibility of outcome (2 cohorts)
• MN-RET reliable quantitative biomarker
• Identification of individual DO mouse resistance and susceptibility genotypes
• Potential to inform genetic epidemiology studies and provide statistical evidence for identifying genes with significant effect size & hypothesis based research
Possible directions

• Repeat BMC modeling with additional exposures at 0.1 ppm and 50 ppm to better define shape of the dose response curve
• Perform 2 year inhalation exposure at 0.1, 1, and 10 ppm to define cancer BMCL and tumor phenotypes
• Stratify BMCL by quartile or decile to estimate the most susceptible subpopulation at risk
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Next steps and future research…..
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