Using Diversity Outbred mice to mimic human population dynamics in susceptibility to environmental chemicals

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Human Health Risk Assessment for Chemicals

Where we almost never have human outcome data and never will

• Many thousands of chemicals in commerce and in the environment
  – Flame retardants, pesticides, dyes, manufacturing catalysts, etc..
  – Arsenic, lead, diesel exhaust, etc…

• Multiple scales and routes of exposure
  – Occupational (long, high dose duration exposure), user (short high dose exposure), communities (long or short, low or high)
• Data needed for risk assessment
  – Hazards – target tissues and outcomes
  – Dose / Potency – what exposure carries an agreeable risk (conservative)
    • Acute versus cumulative
  – Susceptible subpopulations
    • Age
    • Life stage
  – All of these data come from whole animal guideline and in vitro studies
• Gene x environment differences are important

  – 2009: NRC – for risk assessment “attention should be directed to vulnerable individuals and subpopulations that may be particularly susceptible”

  – Understanding influence of genes can inform data-driven uncertainty:
    • Methyl mercury: individual susceptibility can vary 50,000-fold between individuals
    • 5% of population could be 25-fold more susceptible than average
Diversity is rarely incorporated into risk assessment

- NTP Mouse – B6C3F1 hybrid
- NTP Rat – Sprague Dawley – outbred, but with low allelic variation and many IBD regions
- Human cells – Typically from a single donor

- A “feature”, not a “bug”!
- New tools have made it more desirable to consider genetic diversity in chemical safety assessments
Diversity Outbred mice provide genetic diversity

Rationally interbred population that mimics human genetic diversity, but polymorphisms are highly randomized

**Mouse Diversity Panel (MDP)**
- Comprised of dozens of conventional inbred strains
- Each line is genetically distinct
- Many strains have a high degree of genetic relatedness between them, potentially limiting degree of genetic diversity across strains

**Founder Strains:** A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/H1LtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ

**Diversity Outbred mice** are highly genetically diverse, with a randomization of polymorphisms that is superior to human populations

Harrill and McAllister, *Environmental Health Perspectives* 2017
DO Mice: Background of population diversity

DO are a genetic reshuffling of genes from the 8 founder strains

- **129S1/SvImJ** — Sensitive uterine response to oestrogens, high serum cholesterol
- **A/J** — Resistant to cigarette smoke induced emphysema; late onset muscle disease (homozygous mutation)
- **C57BL/6J** — Refractory to many tumors, high susceptibility to diet-induced obesity/T2D, atherosclerosis, high incidence of eye abnormalities
- **NOD/ShiLtJ** — Autoimmune T1D, defects in Ag presentation, impaired wound healing
- **NZO/HILtJ** — Obesity on standard diet and T2D
- **CAST/EiJ** — Highly genetically divergent from other strains, improved neuron axonal regeneration, fast and highly active
- **PWK/PhJ** — Highly genetically divergent from other strains, docile
- **WSB/EiJ** — Highly active, wild temperament, age-related autosomal dominant deafness
Uses for DO mice in Chemical Risk Assessment

Potential Approaches for Population-Based Risk Assessment

Element

Exposure Assessment

- Measure population-wide differences in toxicokinetics to estimate internal dose
- Establish exposure biomarkers for biomonitoring

Harrill and McAllister, *Environmental Health Perspectives* 2017
Usage Case 1: Identify specific polymorphisms associated with xenobiotic toxicity using the DO
DO mice as a model for rare adverse events

Investigating the cause of rare clinical reports of green tea extract herbal supplement liver injury

- Idiosyncratic toxicities have been difficult to study because dose-response relationship is unclear and because MOA is debated
  - Lack of models: DO may fill a gap

Church et al. Food & Chem Toxicol. 2015
Pharmacogenetic analysis of idiosyncratic DILI

Church et al. Food & Chem Toxicol. 2015
**Human translation of GTE PGx risk factors**

Green tea extract (GTE) supplement hepatotoxicity risk alleles in human clinical cases

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>SNP (Array)</th>
<th>Gene name</th>
<th>Chr</th>
<th>P value</th>
<th>Risk/Protective allele</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td>PER3</td>
<td>exm10762</td>
<td>Period circadian clock 3</td>
<td>1</td>
<td>0.004937</td>
<td>T/C</td>
<td>Missense (R/W)</td>
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<td>MFN2</td>
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<td>Mitofusin 2</td>
<td>1</td>
<td>0.0067</td>
<td>A/G</td>
<td>Missense (I/V)</td>
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<tr>
<td>VPS13D</td>
<td>exm16480</td>
<td>Vacuolar protein sorting 13 homolog D (S. cerevisiae)</td>
<td></td>
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<td></td>
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</tbody>
</table>
PGx: chemotherapy-induced neutropenia

N=200-400 DO mice per drug
Single dose level

Implications for precision medicine research & gene x environment analysis

Gatti et al. *The Pharmacogenomics Journal*. 2017
Usage Case 2: Evaluate biomarker performance toward assessing human clinical adverse outcomes
Using a population model to investigate predictive value of novel biomarkers

• Many groups are working on qualifying novel biomarkers into clinical practice

• But, how well will biomarkers identified in genetically limited rodent strains translate to a larger population?

• Do single strain studies prevent us from making human relevant discoveries?

Harrill et al. Tox Sci 2016
Renal Biomarkers to Investigate in DO Mice

• “Gold standard”
  – Creatinine
  – Blood urea nitrogen (BUN)

• Novel proteins qualified for preclinical rat toxicology
  – KIM-1, IP-10, TIMP-1, OPN, Renin, VEGF, NGAL, EGF, clusterin, cysC, albumin, TFF3

• Other urine markers
  – miRNA
Cisplatin Study – Biomarker Performance

Animals: Female DO mice, 8-12 weeks of age
N = 45 cisplatin (5 mg/kg)
N = 45 saline

Goals:
(1) Evaluate performance of conventional kidney injury biomarkers
(2) Evaluate performances of “NEW & IMPROVED” kidney injury biomarkers
(3) Determine whether urinary miRNA biomarkers offer advantages over (1) and (2)
Population dynamics in conventional kidney biomarkers, single cisplatin dose

*P<0.05
Cisplatin induced histopathology
Differential susceptibility

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Vehicle</th>
<th>Cisplatin</th>
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<tbody>
<tr>
<td>Number Examined</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>No Degeneration/Necrosis Present</td>
<td>44</td>
<td>13</td>
</tr>
<tr>
<td>Degeneration (Tubule)</td>
<td>1*</td>
<td>18</td>
</tr>
<tr>
<td>Minimal (Grade 1)</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>Mild (Grade 2)</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Necrosis (Tubule)</td>
<td>-</td>
<td>26</td>
</tr>
<tr>
<td>Minimal (Grade 1)</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>Mild (Grade 2)</td>
<td>-</td>
<td>9</td>
</tr>
</tbody>
</table>

Tubular degeneration or necrosis was not found in vehicle treated animals (excluding 1 animal with minimal severity of tubular degeneration)

No drug-related pathology was found in the liver

*Special thanks to John Seely and NIEHS Pathology
BUN and urinary proteins only elevate when Grade 2 necrosis is present.
The same is true for urinary miRNAs that increase in abundance in response to cisplatin exposure. Elevations in urinary miRNA abundance are only observed at necrosis grade 2.
Even though performance not great at low end of injury spectrum, ROC curves are all significant $p < 0.05$ AND AUROC values 0.71-0.82
Usage Case 3: Tool for population-based estimates of chemical potency for risk assessment

- **BMDL/BMCL**: Statistical lower confidence limit on the BMD/BMC (used as the POD in this case)
- **BMR**: Predetermined change in response rate used to determine the BMD/BMC
- **LOAEL**: Lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects
- **NOAEL**: Highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effects
- **BMD/BMC**: Dose or concentration that produces a predetermined change in response rate of an adverse effect
DO: calculate BMDL for inhaled benzene

Human BMCL$_{10}$ 7.2 ppm (44 subjects), < 1 ppm FX

DO BMCL$_{10}$: 0.205 ppm; B6C3F1 BMCL$_{10}$: 3.12 ppm

French et al. EHP 2015.
More refined recombination structure in the CC/DO result in smaller more refined co-expression networks and correlations among behavioral phenotypes at sample sizes comparable to conventional behavioral QTL mapping studies.

• Predictive Biology has archived hundreds of ES cells from DO mice

• Over 100 male and 100 female DO ES cell lines (representing 200 individual and genetically unique mice) have been differentiated into neural progenitor cells

• Chromosomal aberrations checked by RNA-Seq and Giga MUGA (mouse universal genotyping array)
DNT population responses – differ by chemical and by DO cell line

Wide variability in response across DO cell lines / individuals for a single chemical

Wide variability in response across chemicals for a single DO cell line

Correlation between runs

<table>
<thead>
<tr>
<th>Compound</th>
<th>P values</th>
</tr>
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<tr>
<td>Dieldrin</td>
<td>0.000239</td>
</tr>
<tr>
<td>Ether</td>
<td>0.00071</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.02</td>
</tr>
<tr>
<td>Methyl mercury</td>
<td>0.02</td>
</tr>
<tr>
<td>PBDE</td>
<td>0.0218</td>
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<tr>
<td>Rotenone</td>
<td>0.038</td>
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<tr>
<td>Lead</td>
<td>0.07</td>
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<tr>
<td>BPA</td>
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</tr>
<tr>
<td>Caffeine</td>
<td>0.12</td>
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<tr>
<td>Triphenylphosphate</td>
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<tr>
<td>BPAF</td>
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<tr>
<td>Phthalate</td>
<td>0.3</td>
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<tr>
<td>Dopamine</td>
<td>0.43</td>
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<tr>
<td>Hexachlorophene</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.939</td>
</tr>
</tbody>
</table>
Wide variability in dose responsiveness

Potential Study Outcomes

- Standard uncertainty factor for extrapolating rodent to human NOAEL is 100 – is that protective enough?

- Use DO dose-response data to inform a point of departure for regulatory agencies to set a reference dose (RfD)

Chemical X
BMD – 13.4 uM
Phase 2: Tox21 Cross-Partner Project

**Predictive Biology**
- **Chemical Exposures**
  - Expose DO NPCs and fix cells

**NTP, EPA, & FDA/NCTR**
- **Dose Concentration Selection**
  - Input into study design, chemical selection, and target doses

**NCTR/FDA**
- **High Content Imaging**
  - Label targets with fluorescent probes
  - Scan/Image cells
  - Image analysis / quantify fluorescent intensity and morphometry

**NIEHS/NTP**
- **Data Analysis and BMD Assessment**
  - Calculate BMD/BMDL for each chemical/sex and for each endpoint
  - Determine relative sensitivity of each HCA probe and DO cell line
  - Determine sex differences in susceptibility for each compound

**Cross-Partner Data Contextualization (NTP/FDA)**
Model Evaluation: Compare BMDs calculated using DO NPCs to *in vivo* data collected by NTP and other stakeholders; determine concordance with human epidemiological data where available, compare variability with other DNT screens under development (human iPSC neurite outgrowth, zebrafish, others)
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