Molecular mechanisms for persistence of the effects of developmental toxicants: The fetal basis of adult disease/dysfunction, and potential for transgenerational inheritance

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Acknowledgements

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Rebekah Klingler
Mathew Pickens
Thomas Kalluvila
Francisco Mora
Abby Debofsky

UWM Zilber School of Public Health
Michael Laiosa
Kurt Svoboda
Peter Tonellato

American University
Victoria Connaughton

University of Toledo
Fred Williams

Washington State University
Mike Skinner

Supported by:
IHS/NIH Great Lakes Native American Research Center for Health
National Institute of Environmental Health Sciences
University of Wisconsin-Milwaukee Children’s Environmental Health Sciences Core Center
USEPA Great Lakes Restoration Initiative
Barker Hypothesis

The proposition that a baby's nourishment in utero and during infancy determines the subsequent development of risk factors such as high blood pressure, blood clotting biochemistry and glucose intolerance and is thus a major determinant of coronary heart disease later in life.


Developmental Origins of Health and Disease
Laboratory Focus

Environmental Chemicals

Mother-offspring transfer

Neurological deficits in offspring

F2, F3, F4...
For lipophilic chemicals with a long-half life:

Yolk partitioning upon direct exposure (4-24 hpf), with subsequent distribution to embryo during growth and yolk utilization, creates a developmental exposure similar to that of mammals WITHOUT the influences of maternal metabolism.
Acoustic/Vibrational Startle Reflex in Zebrafish

• Simple reflex
  Stimulus (acoustic/vibrational, touch, visual), Receptors, Mauthner cell, Interneurons, Trunk muscles

• C-start
  Latency of response
  Angle of flexion
  Escape velocity
  Habituation
Adult Visual Startle Response
Adult Visual Startle—Direct Exposure (4-24 hpf)

Visual Startle Responses in 5 Minutes

Weber et al., Physiol Behav 2008.
Possible Mechanisms for Deficit

Sites of concern:
- Retina, Optic Nerve, Optic Tectum
- Locomotor Pathways

- Permanent structural damage during development?
- Mistakes in neuronal development?
- Permanent changes in neuronal function?

untreated

0.3µM MeHg
Retinal Electrophysiology

http://www.pdn.cam.ac.uk/staff/harris/cell.jpg
Effects on Adult Retinal Potassium Currents

Delayed rectifying ($I_K$) current is enhanced in adult zebrafish following developmental exposure to MeHg.

Weber et al., Physiol Behav 2008.
Effects on Spinal Motorneurons

Tg (*Isl1:gfp*)  
0.1 µM MeHg

And they are HYPERACTIVE
Spatial Alternation Task

FOOD or

<table>
<thead>
<tr>
<th>Treatment</th>
<th>trials to 75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>13.9</td>
</tr>
<tr>
<td>Ethanol 10mM</td>
<td>21.7*</td>
</tr>
<tr>
<td>Ethanol 30mM</td>
<td>27.4*</td>
</tr>
<tr>
<td>Pb 10µM</td>
<td>31.2*</td>
</tr>
<tr>
<td>Pb 30µM</td>
<td>none*</td>
</tr>
<tr>
<td>MeHg 0.1µM</td>
<td>none*</td>
</tr>
<tr>
<td>MeHg 0.03µM</td>
<td>none*</td>
</tr>
</tbody>
</table>
Mercury-Induced Transgenerational Inheritance of Abnormal Phenotypes
Visual Startle in Adult F2 Lineages

F2 Visual Startle Responses in 5 Minutes

Carvan et al., PLoS ONE 2017
Effects on Retinal Potassium Currents in F2 Lineages

Carvan et al., PLoS ONE 2017

Figure 4. Change in peak amplitude of $I_K$ current recorded from bipolar cells in retinas exposed to various concentrations of MeHg. Representative current traces are given at the top. The graph at the bottom plots mean peak currents elicited at different voltage steps from a holding potential of -60mV.
Spontaneous Activity in F2 Lineages

They’ve lost their “glide”

Carvan et al., PLoS ONE 2017
Distribution of Phenotypes in F2 Lineages

Independent inheritance

Carvan et al., PLoS ONE 2017
## Independent inheritance

### Supplemental Table S2: Evaluation of expected versus observed inheritance of neurobehavioral phenotypes.

<table>
<thead>
<tr>
<th>F2 lineage (nM)</th>
<th>n</th>
<th>Neither Phenotype</th>
<th>Visual deficit</th>
<th>Hyperactivity</th>
<th>Both Phenotypes</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Expected</td>
<td>Observed</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>87% (13)</td>
<td>7% (1)</td>
<td>7% (1)</td>
<td>0% (0)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>5% (1)</td>
<td>65% (13)</td>
<td>65% (13)</td>
<td>42% (8)</td>
<td>35% (7)</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>6% (1)</td>
<td>93% (14)</td>
<td>38% (6)</td>
<td>35% (6)</td>
<td>31% (5)</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>6% (1)</td>
<td>72% (13)</td>
<td>61% (11)</td>
<td>44% (8)</td>
<td>39% (7)</td>
</tr>
<tr>
<td>30</td>
<td>17</td>
<td>6% (1)</td>
<td>71% (12)</td>
<td>71% (12)</td>
<td>50% (9)</td>
<td>47% (8)</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>0% (0)</td>
<td>93% (14)</td>
<td>60% (9)</td>
<td>56% (8)</td>
<td>53% (8)</td>
</tr>
</tbody>
</table>

\( \chi^2 = 0.067 \)  
\( \text{df} = 4 \)  
\( p = 0.999 \)
Mechanism? Epigenetic Inheritance

DNA methylation epimutations have been shown to be heritable and associated with transgenerational inheritance in several species.

## Transgenerational Inheritance in Zebrafish

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Citation</th>
<th>Phenotypic endpoint(s)</th>
<th>Epigenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCDD</td>
<td>Baker TR et al. 2014</td>
<td>Skeletal development, sex ratio, male-mediated reproduction</td>
<td>None</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Lombó et al. 2015</td>
<td>Heart failure</td>
<td>Global DNAmet in testes, sperm</td>
</tr>
<tr>
<td>Akhter, et al. 2018</td>
<td></td>
<td>Reproductive abnormalities</td>
<td>None</td>
</tr>
<tr>
<td>Methylmercury</td>
<td>Carvan et al. 2017</td>
<td>Neurobehavior, bipolar cell electrophysiology</td>
<td>MeDIPseq in sperm</td>
</tr>
<tr>
<td>Mono(2-ethylhexyl) phthalate</td>
<td>Kamstra et al. 2017</td>
<td>None</td>
<td>Locus-specific methylation (6 and 2, respectively)</td>
</tr>
<tr>
<td>5-azacytidine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Knecht et al. 2017</td>
<td>Neurobehavior, BMI, heartbeat, mitochondrial function</td>
<td>Global DNAmet</td>
</tr>
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</table>
### Differentially Methylated Regions in F0 Sperm

#### Number of DMRs using different EdgeR p-values cut-off thresholds

<table>
<thead>
<tr>
<th>p-value</th>
<th>allWindow</th>
<th>twoWindow</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^{-3}$</td>
<td>10125</td>
<td>2966</td>
</tr>
<tr>
<td>$1 \times 10^{-4}$</td>
<td>3005</td>
<td>1171</td>
</tr>
<tr>
<td>$1 \times 10^{-5}$</td>
<td>1383</td>
<td>634</td>
</tr>
<tr>
<td>$1 \times 10^{-6}$</td>
<td>811</td>
<td>413</td>
</tr>
<tr>
<td>$1 \times 10^{-7}$</td>
<td>533</td>
<td>291</td>
</tr>
</tbody>
</table>
Differentially Methylated Regions in F2 Sperm

Number of DMRs using different EdgeR p-values cutoff thresholds

<table>
<thead>
<tr>
<th>p-value</th>
<th>allWindow</th>
<th>twoWindow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x10^{-3}</td>
<td>22877</td>
<td>8370</td>
</tr>
<tr>
<td>1x10^{-4}</td>
<td>8499</td>
<td>3429</td>
</tr>
<tr>
<td>1x10^{-5}</td>
<td>4093</td>
<td>1771</td>
</tr>
<tr>
<td>1x10^{-6}</td>
<td>2307</td>
<td>985</td>
</tr>
<tr>
<td>1x10^{-7}</td>
<td>1414</td>
<td>617</td>
</tr>
</tbody>
</table>

Chromosomal Plot

Carvan et al., PLoS ONE 2017
RNAseq Analysis F2 Adults

Dysregulated KEGG pathways

Brain

Retina

unpublished

FDR < 0.05
Linking DOHaD, Epigenetics, Transcriptomics

Relationship between the germline epigenome and that of somatic cells associated with neurobehavioral defects (anatomy, physiology, MeDIPseq, RNAseq)

unpublished
Linking Epigenetics and Transcriptomics

We can do gene expression and DNAmet in the same cell prep

unpublished
Powerful System for DOHaD, Generational Effects

**Strengths**

- Genome resources
- Most cell/molecular pathways similar to humans
  - Complex behaviors
- NOT inbred
- Few limitations on replication
  - Large clutch size
- GFP-labeled lines for most cell types
- Can complement (limit) the use of mammals in research

**Weaknesses**

- They are not mammals
  - No mammary tissue
  - No lungs
- Small tissues can be limiting
  - Analytical chemistry
  - Biochemistry
  - Physiology
The Bemidji Statement on Seventh Generation Guardianship

"The first mandate...is to ensure that our decision-making is guided by consideration of the welfare and well being of the seventh generation to come."

http://www.sehn.org/bemidjistatement.html
## Methylmercury Treatments

### Total Hg Analysis and Evaluation of Dosimetry

<table>
<thead>
<tr>
<th>Media, Nominal (nM)</th>
<th>Media, Measured (nM)</th>
<th>Embryo, Measured (ppb)</th>
<th>Similar values in human cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.15 ± 0.02</td>
<td>5.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.5 ± 0.4</td>
<td>19.4 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.9 ± 0.2</td>
<td>51 ± 3.5</td>
<td>1% Lake Superior</td>
</tr>
<tr>
<td>10</td>
<td>10 ± 0.05</td>
<td>257 ± 10</td>
<td>Highest Lake Superior</td>
</tr>
<tr>
<td>30</td>
<td>32 ± 0.6</td>
<td>836 ± 68</td>
<td>Minamata, Japan</td>
</tr>
<tr>
<td>100</td>
<td>104 ± 0.4</td>
<td>2819 ± 152</td>
<td></td>
</tr>
</tbody>
</table>

Carvan et al., PLoS ONE 2017