Of Mice and Men: Using Animal Models to Study Gene-Environment Interactions

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Courtesy of Jeff Murray
How Does One Study Gene-Environment Interactions that Governing Susceptibility to Birth Defects?
Embryonic Development is Determined by Maternal Lifestyle Choices and Genetic Factors

THE MOTHER IS THE INTRA-UTERINE ENVIRONMENT OF THE DEVELOPING EMBRYO AND FETUS

- Nutrition
- Medication
- Immune Response
- Genetic factors
- Lifestyle
- Chemical exposures
- Smoking
- Alcohol
- Drugs
- Health
Environment is defined as the body’s internal chemical environment.

Exposure is defined as the amounts of biologically active chemicals in this internal environment.
Possible Genetic and Environmental Interactions

- gene-gene
- gene-environment
- environment-environment
- g x g x e x e...........
Gene-Environment Interactions-Maternal Smoking, Folate Status, and Orofacial Clefts

- population-based case-control study
  California, n=548,844 1987-89 births
- cases - isolated CLP
  n=244/318 eligible mothers interviewed and infants genotyped
- controls - randomly selected, 588/652 eligible mothers interviewed and infants genotyped
- DNA from newborn blood samples
Maternal characteristics of isolated CLP cases and nonmalformed controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=244)</th>
<th>Controls (n=588)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multivitamin Use</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>29.5%</td>
<td>18.5%</td>
</tr>
<tr>
<td>Use -1 through +2</td>
<td>68.4%</td>
<td>80.4%</td>
</tr>
<tr>
<td><strong>Cigarette Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>66.8%</td>
<td>76.2%</td>
</tr>
<tr>
<td>Yes -1 through +2</td>
<td>32.8%</td>
<td>23.3%</td>
</tr>
</tbody>
</table>

Shaw et al., Am J Epidemiol. 2005;162(12):1207-14
Nitric Oxide Synthase

- \( NOS3 \) variants influence (raise) homocysteine concentrations
- smoking compromises \( NOS3 \) activity
- folate intake influences (lowers) homocysteine concentrations
- is clefting risk from \( NOS3 \) variants modified by smoking and further modified by vitamin intake (folic acid)?
Genotyping

• 3 SNPS, A922G, C690T, and G894T

• multilocus allele-specific hybridization assay

• Roche Molecular Systems

• panel of 32 SNPs

• all 3 SNPs consistent with Hardy-Weinberg equilibrium in controls
### NOS3 C690T genotypes, maternal smoking, maternal vitamin use, and CLP risks

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Smoking</th>
<th>Vitamin Use</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variant</td>
<td>Yes</td>
<td>No</td>
<td>4.7</td>
<td>0.9-26.8</td>
</tr>
<tr>
<td>Variant</td>
<td>Yes</td>
<td>Yes</td>
<td>2.0</td>
<td>0.7-5.8</td>
</tr>
<tr>
<td>Wildtype</td>
<td>Yes</td>
<td>No</td>
<td>3.1</td>
<td>1.6-6.0</td>
</tr>
<tr>
<td>Wildtype</td>
<td>Yes</td>
<td>Yes</td>
<td>1.7</td>
<td>1.1-2.6</td>
</tr>
<tr>
<td>Wildtype</td>
<td>No</td>
<td>Yes</td>
<td>Ref</td>
<td>-----</td>
</tr>
</tbody>
</table>

Shaw et al., *Am. J. Epidemiol.* 162:1207-14, 2005
Neural Tube Defects

- 250-300,000 NTD births annually worldwide; 3,000 in US
- Result in lifelong disability
  - Problems with bladder, bowel, and sexual function
  - Learning and developmental problems
  - Orthopedic problems
- Some NTDs are preventable—Approx. 20% reduction since folate fortification in US
Neural Tube Defects

neural plate       neural folds       neural tube

Anencephaly

Spina Bifida
NTDs are Complex Traits

- They have a strong genetic component
- They also require a significant environmental interaction in order to express the abnormal phenotype
maternal characteristics as well as exposures that influence the *in utero* environment of the developing embryo

- **Established risk factors**
  - maternal folate status
  - *pre-gestational diabetes*
  - maternal use of anti-epileptic drugs
  - maternal obesity

- **Compelling evidence**
  - maternal vitamin B12 status
  - maternal hyperthermia

- **Proposed, but unconfirmed**
  - exposure to fumonisins
  - pesticides
  - hazardous waste sites
While No Doubt True....

“The proper study of mankind is man.” Alexander Pope

Courtesy of Laura Mitchell
IN EXPERIMENTAL DESIGNS TO TEST GENETIC SUSCEPTIBILITY

ENVIRONMENT IS HELD CONSTANT WHILE MANIPULATING THE GENOTYPE OF THE EXPERIMENTAL ORGANISM
FOR EXPERIMENTS CONCERNING GENETIC SUSCEPTIBILITY TO TERATOGENESIS, THE MOUSE IS THE IDEAL EXPERIMENTAL ORGANISM

• >22,000 individual genes have been identified and mapped with the completion of the mouse genome project

• >4000 genetically engineered mouse lines now exist

• >250 inbred mouse strains exist
图1 同型半胱氨酸代谢

**CBS**: 胱硫醚β合成酶

**MTR**: 蛋氨酸合成酶

**MTHFR**: 亚甲基四氢叶酸还原酶
Modelo de ratón knockout para un gen transportador de ácido fólico.

Receptor de folatos

Folr1
Folr2
RFC1
PCFT
NTDs With Folate Supplementation
(Folr1⁻/⁻, E18, 5M-THF, 12.5mg/kg)
Restoring Folate Concentrations in Light of Gene Defect Restores Normal Phenotype

Percentage

<table>
<thead>
<tr>
<th>Daily Dose of Folinic Acid (mg/kg)</th>
<th>NTDs</th>
<th>normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>6.25</td>
</tr>
<tr>
<td>6.25</td>
<td>76.5</td>
<td>23.5</td>
</tr>
<tr>
<td>12.5</td>
<td>77.8</td>
<td>22.2</td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
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</table>

Restoring Folate Concentrations in Light of Gene Defect Restores Normal Phenotype
Folic Acid Responsive Targets (FARTs)
Pollutants in Areas Associated With High NTD Prevalence

- Fine particulate air pollution (<2.5 µm; PM$_{2.5}$)
- Arsenic
- Carbon disulphide
- Cadmium
- Lead
- TCDD
Maternal Obesity is an NTD Risk Factor

- Obesity is an Inflammatory Disease

2/3rds of US women overweight = 1.22 OR
1/3rd of US women are obese = 1.7 OR
7% of US women are morbidly obese = 3.1 OR

Inflammatory markers increase on high fat diets in mice.
Arsenic-Induced NTDs in Folr2 Mice

Folr2 Nulls are Highly Susceptible to Arsenic-Induced NTDs

Gene X Environment X Environment Interaction

Folb2+/-  Folb2-/-  Folb2+/-  Folb2-/-
Folate Replete Diet (FRD)  Folate Deficient Diet (FDD)

Exencephaly (%)

<table>
<thead>
<tr>
<th></th>
<th>Water</th>
<th>Arsenate</th>
<th>Water</th>
<th>Arsenate</th>
<th>Water</th>
<th>Arsenate</th>
<th>Water</th>
<th>Arsenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folb2+/-</td>
<td>0.0</td>
<td>24.0</td>
<td>0.0</td>
<td>40.6</td>
<td>0.0</td>
<td>25.7</td>
<td>1.0</td>
<td>64.0</td>
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MATERNAL HYPERThERMIA AS A NEURAL TUBE DEFECT CAUSING TERATOGEN

• Risks associated with increase in core temperature above 38.9°C

• Could be occupational or secondary to a disease process
Limited level of technology needed to study hyperthermia*

- Water bath
- Ring stand and clamp
- Thermometer
- 50ml centrifuge tube
- Redwood decking (optional)
SWV HYPERTHERMIA TREATMENT

HEAT CURVE

Temperature °C

Time (minutes)
Good Mouse
Bad Mouse

Exencephaly, cleft face
Hyperthermia-Induced Exencephaly

% Exencephaly

Strain Comparison

DBA  C57  SWR  Balb/c  LM/Bc  SWV
Mouse Models of Valproic Acid-Induced Neural Tube Defects

Treat Pregnant Dams From Multiple Inbred Mouse Strains at E8.5 with 600 mg/kg VPA

Collect Fetuses at E15.5 and Examine for Presence of NTDs
VALPROIC ACID TREATMENT

% VPA induced Exencephaly

Strain Comparison
How Does One Find Modifying Genes to Explain Genetic Susceptibility to VPA-Induced NTDs?

- Genetic Linkage Analyses
- Whole Genome Wide Analyses for Modifying Genes
  - Completed for SWV and C57
  - Located 1CM region on Chromosome 7
  - Multiple candidate genes localized to this region
NTD Response Rates

Breeding Scheme

Strain %NTDs

F1a = SWV x C57
F1b = C57 x SWV
BC1a = F1a x SWV
BC1b = SWV x F1a
Using Mouse Genetics to Find Modifying Genes to Explain Genetic Susceptibility to VPA-Induced NTDs?

Use of Consomic (Chr. Substitution) Mouse Strains

- Treat pregnant mice with Chr. 7 from VPA sensitive strain (A/J) placed on resistant background (C57) with VPA and collect fetuses
- Confirms Chr. 7 as site of sensitivity genes
- Screen candidate genes for SNPs associated with increased susceptibility

Pregnancy Outcome with Gestational VPA Exposure

<table>
<thead>
<tr>
<th>Strain</th>
<th>Abnormal</th>
<th>Normal</th>
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</thead>
<tbody>
<tr>
<td>A/J</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>C57BL/6J-Chr7A/J</td>
<td>10.0%</td>
<td>90.0%</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>20.0%</td>
<td>80.0%</td>
</tr>
</tbody>
</table>

- Tnrc6a
- Prkcb1
- Rbbp6
# Linkage Region

<table>
<thead>
<tr>
<th>Location</th>
<th>P(two-tailed)</th>
<th>Chi²</th>
<th>Genotype</th>
<th>Chrm</th>
<th>Bp</th>
<th>-bp</th>
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<tbody>
<tr>
<td>D7Mit220</td>
<td>38.3</td>
<td>2.00E-04</td>
<td>15.47</td>
<td>88/43</td>
<td>7</td>
<td>111543239 111543373</td>
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<tr>
<td>D7Mit285</td>
<td>44.8</td>
<td>2.00E-06</td>
<td>23.1</td>
<td>93/38</td>
<td>7</td>
<td>129629838 129629943</td>
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<tr>
<td>D7Mit101</td>
<td>45.9</td>
<td>2.00E-05</td>
<td>18.34</td>
<td>90/41</td>
<td>7</td>
<td>132776553 132776641</td>
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<tr>
<td>D7Mit105</td>
<td>49.2</td>
<td>5.00E-05</td>
<td>16.87</td>
<td>89/42</td>
<td>7</td>
<td>135707912 135708169</td>
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</table>

![Genetic Map of Chromosome 7](image)

Chrm 7
ACSMs
Acyl-CoA Synthetase Medium-Chain Family Members

<table>
<thead>
<tr>
<th>Chr</th>
<th>bp</th>
<th>-bp</th>
<th>Sym</th>
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<tbody>
<tr>
<td>7</td>
<td>126750540</td>
<td>12680602</td>
<td>9</td>
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<td>7</td>
<td>126705007</td>
<td>12674011</td>
<td>5</td>
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<td>7</td>
<td>126904437</td>
<td>12693102</td>
<td>7</td>
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<tr>
<td>7</td>
<td>126833540</td>
<td>12685808</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>126669779</td>
<td>12668687</td>
<td>4</td>
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Significance (P) vs. bp
Next Steps to Understanding Genetic Basis of Susceptibility to VPA-Induced NTDs

- Deep DNA Resequencing of Relevant Regions of the ACSM gene family in SWV and C57 Mice

- Development of ACSM Knockout Mouse Models to be Challenged with VPA Treatment

- Have Obtained ES clones for ACSM4 and Blastocyst Injections are in Progress

- Human Patients From NEAD Study Exposed to VPA in utero with Variable Outcomes will be sequenced for variants in the ACSM gene family

- Multiple candidate genes localized to this region
“NTDs are caused by a little bit of this and a little bit of that”

Clarke Fraser
09/12/09
COLLABORATING INVESTIGATORS

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University of Queensland
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Dr. Trent Woodruff

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University of Texas-Austin
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Dr. Dean Appling
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US Environmental Protection Agency
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