The Josef Warkany Lecture

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Disclosure

I have no financial or other interests that pose a conflict of interest.

My research is funded by the Canadian Institutes of Health Research (CIHR).
50 Years of Progress in Understanding the Causes of Three Common Birth Defects

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McGill Univ.

Jim Miller*

UBC

Muriel Harris

Fred Biddle

DMJ, MSC, gene–riboflavin deficiency interaction induced cleft palate in chicks

DMJ, PhD, Genes and 6–AN induced cleft lip

DMJ, postdoc, Genes, otocephaly

UBC

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Jan Friedman*

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McGill Univ.

DMJ, faculty, Genes, epigenetics, cleft lip, NTD, eyelids, thyroxine, cortisone

Kathy Sulik*

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* Former President of Teratology Society
50 Years of Progress in Understanding the Causes of Three Common Birth Defects

- Cleft lip and palate
- Neural tube closure defects
- Gastrochisis

Genes/Environments: relative roles in etiology of birth defects

- GENES
- ENVIRONMENTS

Mostly genetic
Mostly environmental
Genes/Environments: relative roles in etiology of birth defects

- Achondroplasia
- Down Syndrome
- Cystic Fibrosis
- Cleft lip
- NTD
- Thalidomide
- Accutane
- Gastrochisis
**Gastroschisis**

Herniation of loop of gut

Adapted from: Brewer and Williams, 2004. *Bioessays 26:1307*

No mention of genetics or environmental factors.

Usually an isolated abnormality

Incidence 1/17,000 births

Long known. Cites article by Calder (1733)

Distinguished from omphalocele
Gastrochisis rising frequency. Rate per 100,000 births.


Gastrochisis mouse models?

- 40 mouse gene mutants have body wall defects.
- Only one looks like human gastrochisis, anatomically: Aebp1 (ACLP). This codes a secreted protein in collagen–rich tissues in embryogenesis.

WHAT IS NEEDED.

- Better pictures. Most gene or teratogen studies do not describe the defect clearly.
- Histology of the edges of the “hole”. Did it tear? Did a patch of cells die?
- Embryonic forensics. What went wrong first?
Cleft lip and palate

Approximately 1 in 1000 births. Populations differ.

Human CLP photos. See textbooks, such as Langman’s Medical Embryology edited by Sadler.


Cleft lip and palate: >50 years ago
Cleft lip and palate: 50 years ago


CLP 1970's

Carter, Falconer, Fraser: multifactorial models

Three facial prominences of different origins have to grow together, meet and fuse in a narrow time widow.
GD 11 embryos in the A/WySn mouse strain

Multifactorial CLP

CLP 2010: Mouse CLP mutants

10 identified genes
CLP genes: expression domains to be studied. Limited information.

- **GENES:**
  - Bmp4
  - Bmpr1a
  - Sox11
  - Tcfap2a
  - Wnt9b
  - Lrp6
  - Tp63
  - Alk5
  - Rspo2

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**Mutation at Wnt9b gene**

A transposon of the IAP class has inserted at the 3’ end of the Wnt9b gene.

- The IAP at this location is present in all ALL A/WySn individuals.
- The IAP is not present at this location in other strains.

Ref: Juriloff et al 2005, Birth Defects Res A
CLP 2010: Epigenetic Mouse *Wnt9b* mutant model

**Methylation of the IAP at the Wnt9b gene**

Cleft lip embryos have a poorly methylated IAP.

<table>
<thead>
<tr>
<th>Periconceptional agent</th>
<th>Effect on CLP rate</th>
<th>Odds Ratio or Relative Risk</th>
<th>Example of References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal heavy smoking</td>
<td>Increased</td>
<td>1.8</td>
<td>Honein et al., 2007</td>
</tr>
<tr>
<td>Maternal obesity (BMI)</td>
<td>Increased</td>
<td>1.2</td>
<td>Stothard et al., 2009</td>
</tr>
<tr>
<td>Maternal folic acid supplement</td>
<td>Decreased</td>
<td>0.5</td>
<td>Badovinac et al., 2007</td>
</tr>
</tbody>
</table>
CLP human genes 2010: examples

<table>
<thead>
<tr>
<th>Positional cloning</th>
<th>Population</th>
<th>Candidate /linkage approach</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVRL1</td>
<td>Venezuela</td>
<td>IRF6</td>
<td>Many (~10% of risk)</td>
</tr>
<tr>
<td>GWAS* on SNPs</td>
<td>8q24, rs987525</td>
<td>TGFB3</td>
<td>China, Chile...</td>
</tr>
<tr>
<td>17q22, rs227731</td>
<td>European</td>
<td>MTHFR</td>
<td>China...</td>
</tr>
<tr>
<td>10q25.3, rs7078160</td>
<td>European</td>
<td>MSX1</td>
<td>Various</td>
</tr>
<tr>
<td>MAFB, rs13041247</td>
<td>Asian</td>
<td>PTCH1</td>
<td>Ireland ...</td>
</tr>
<tr>
<td>ABCA4, rs560426</td>
<td>Asian</td>
<td>FOXE1</td>
<td>Various</td>
</tr>
</tbody>
</table>

CLP 2010 –still multifactorial

Case 1 genes
Case 2 genes + environments
Case 3 other genes + other environments
Case 4 genes + teratogen
Case 5 genes + environment + epigenetics
Case 6 environment + teratogen + epigenetics
Causality???

Not really

NTD: What are they?

Image of types of NTD and their relation to neural tube closure events.

Copyrighted. See original paper cited at right.

From: Botto et al., 1999, NEJM 341:1509
Little was known about:
- Genetics
- Developmental etiology
- Environmental factors

Aspects that had been noted:
- Season effects
- Urban/Rural effects
- Elevated recurrence risk in families
- 70% of anencephalics are female

1953 MacMAHON, PUGH, and INGALLS, Brit. J. prov. soc. 7, 211–219

1977 Smithells RW, Sheppard S, Schorah CJ. Folates and the fetus. Lancet
To settle the debate...


<table>
<thead>
<tr>
<th>Group *</th>
<th>NTD / Informative pregnancies</th>
<th>NTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid (4 mg)</td>
<td>2/298</td>
<td>1%</td>
</tr>
<tr>
<td>Folic Acid (4 mg) plus vitamins</td>
<td>4/295</td>
<td></td>
</tr>
<tr>
<td>Neither</td>
<td>13/300</td>
<td>3.5%</td>
</tr>
<tr>
<td>Vitamins</td>
<td>8/302</td>
<td></td>
</tr>
</tbody>
</table>

TOTAL = 1195 pregs

RR = 0.28 (95% CI= 0.12–0.71); indicates 72% of NTDs prevented by folic acid.

*Periconceptional until week 12

<table>
<thead>
<tr>
<th>Group*</th>
<th>NTD/informative pregnancies</th>
<th>P = 0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid (0.8 mg), vitamins, minerals</td>
<td>0/2104</td>
<td></td>
</tr>
<tr>
<td>Minerals</td>
<td>6/2052</td>
<td></td>
</tr>
</tbody>
</table>

• A lower dosage of folic acid can reduce the rate of occurrence of NTD in mothers who have not had an NTD in a previous pregnancy.
• The MRC study indicated that the other vitamins and minerals in the mix may not have had a role.

* Preconceptional until 3rd month

NTD: Folic acid fortification of flour

Examples:
Canada, USA, yes. England, Finland, no.

Canadian study. 1,900,000 births. P < 0.0001

Spina bifida per 1,000 births

Canadian study. 1,900,000 births. P < 0.0001
NTD: USA folic acid fortification

Adapted from: Honein et al., 2001, JAMA 285:2981-2986

NTD per 100,000 births

NTD 2010 and Folate

- Heterogenous causes of NTD
- Not all NTD can be prevented by folic acid
- Concept of “folate–resistant” NTD
- Can other environmental factors prevent some of the remainder?
- Examples: Inositol, Choline, reduced obesity.
Lack of statistical power in most studies. Only 9% of studies have at least 500 cases.

Need 1,000 cases and 1,000 controls to detect a 2X effect of a gene 80% of the time. (Au et al., 2010).

Need GWAS.

From candidate approaches on small samples there are about 25 genes that might be NTD factors. But this is “searching in the dark under the lampost”? Better to do unbiased GWAS; if these are real factors, they will show up there too.

Ref: Au et al., 2010 for list of genes.
The NTD gene search has been highly biased towards obvious folate and methylation pathway genes for spina bifida and anencephaly.

The results are weak. DHFR, MTHFD1, MTHFD1L, MTHFR, TYMS, BHMT, MTRR are possibles.

Ref: Au et al 2010

### NTD mouse mutants

<table>
<thead>
<tr>
<th>NTD type</th>
<th>Number of mutant genes</th>
<th>Functions of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exencephaly (= anencephaly)</td>
<td>145</td>
<td>Many functions. e.g. signaling pathways, actin function, primary cilia, apoptosis, chromatin structure, inositol metabolism</td>
</tr>
<tr>
<td>Spina bifida</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Spina bifida with exencephaly</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Craniorachischisis</td>
<td>14</td>
<td>Planar Cell Polarity; Convergent extension</td>
</tr>
</tbody>
</table>

Harris and Juriloff, 2007 & 2010
Mouse mutants with folate pathway gene mutations that survive to neural tube closure mostly do not have NTDs

<table>
<thead>
<tr>
<th>Genes</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cbs</em> normal neural tube</td>
<td><em>Mthfr</em> normal neural tube</td>
</tr>
<tr>
<td><em>Folr2</em> normal neural tube</td>
<td><em>Mtrr</em> hypomorph normal neural tube</td>
</tr>
<tr>
<td><em>Mthfd1</em> normal neural tube</td>
<td><em>Shmt1</em> normal neural tube</td>
</tr>
<tr>
<td><em>Mthfd2</em> normal neural tube</td>
<td><em>Slc46a1</em> (PCFT) normal neural tube</td>
</tr>
<tr>
<td><em>Folr1</em> + folic acid NTD</td>
<td><em>RFC1</em> + folic acid NTD</td>
</tr>
</tbody>
</table>

Harris and Juriloff, 2007 & 2010

Mouse NTD mutants and folic acid

<table>
<thead>
<tr>
<th>Mutants that respond to folic acid</th>
<th>Function of gene</th>
<th>Mutants that do not respond to folic acid</th>
<th>Other effective agents</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cart1</em></td>
<td>Transcription</td>
<td><em>Axd</em></td>
<td>methionine</td>
</tr>
<tr>
<td><em>Cited2</em></td>
<td>Transcription coactivator</td>
<td><em>Grhl3</em> (ct)</td>
<td>Inositol, retinoic acid</td>
</tr>
<tr>
<td><em>Lrp6 (Cd)</em></td>
<td>Wnt signaling</td>
<td><em>Fkbp8</em></td>
<td>--</td>
</tr>
<tr>
<td><em>Gcn5</em></td>
<td>Chromatin structure</td>
<td><em>Map3k4</em></td>
<td>--</td>
</tr>
<tr>
<td><em>Pax3 (Sp)</em></td>
<td>Transcription</td>
<td><em>Nog</em></td>
<td>--</td>
</tr>
<tr>
<td><em>Folr1</em></td>
<td>Folate receptor</td>
<td></td>
<td></td>
</tr>
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Harris and Juriloff, 2007 & 2010
Recent report from China that NTD cases have hypomethylated DNA.


Most of what we know is based on mouse.

Exencephaly in Embryo and fetus
Neural fold elevation zones and fusion initiation sites.
Sites of common NTDs relate to elevation zones.

Day 9 mouse embryo. Mouse fetus. Human fetus.

All mouse mutants examined have failure of neural fold elevation, and therefore the species difference in first contact sites seems irrelevant.

The location of first contact and fusion of cranial neural folds differs between normal mouse strains that do not have NTD.

These normal variants may affect liability to teratogens that cause NTD.

Source: Juriloff et al., Teratology, 1991
 Median hinge point (MHP)
Anterior spine

Lateral hinge point (LHP).
Caudal spine and the head.
This is what seems to fail in
exencephaly and spina bifida

Ref: Copp & Greene. J Pathol 2009

Lack of MHP and wide short neural tube.
Due to lack of convergent extension.
Leads to craniorachischisis.

**Neural tube closure 2010**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>genes</td>
<td>genes + environments</td>
<td>other genes + other environments</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>genes + teratogen</td>
<td>genes + environment + epigenetics</td>
<td>environment + teratogen + epigenetics</td>
</tr>
</tbody>
</table>

**CLP and NTD 2010 – multifactorial**

How do we address causality in this kind of system???
**Genes/Environments**

**Lewontin concepts:**
Genes act in the context of environments.
A gene gives different effects in different environments.
Environments give different effects in the context of different genes.

**INTERFACE:**
- Genes plus environments
- Genes increase risk
- Environments increase risk
- Specific interactions increase risk

**WE CAN REDUCE THE RISK:**
- Remove the “bad” environments
- Improve other environment
- Compensate for gene effects
- Fix epigenetic lesions?