The Josef Warkany Lecture

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Disclosure

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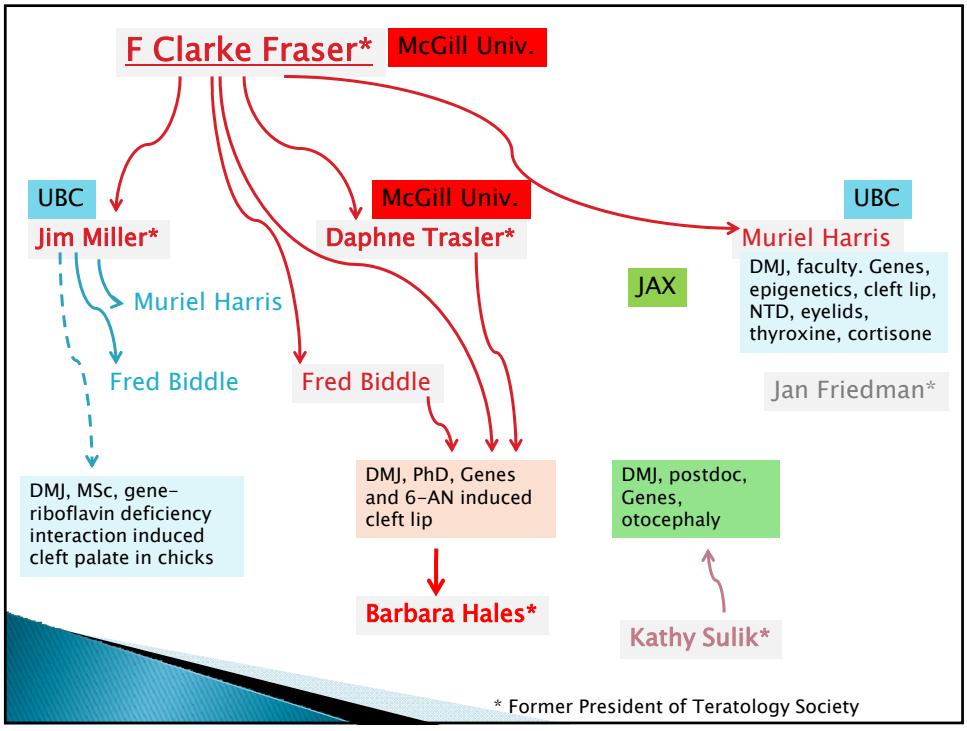
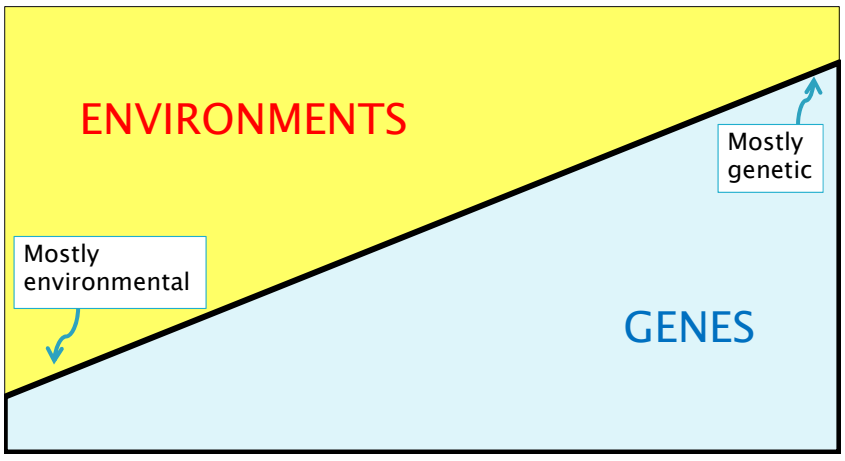
50 Years of Progress in Understanding the Causes of Three Common Birth Defects



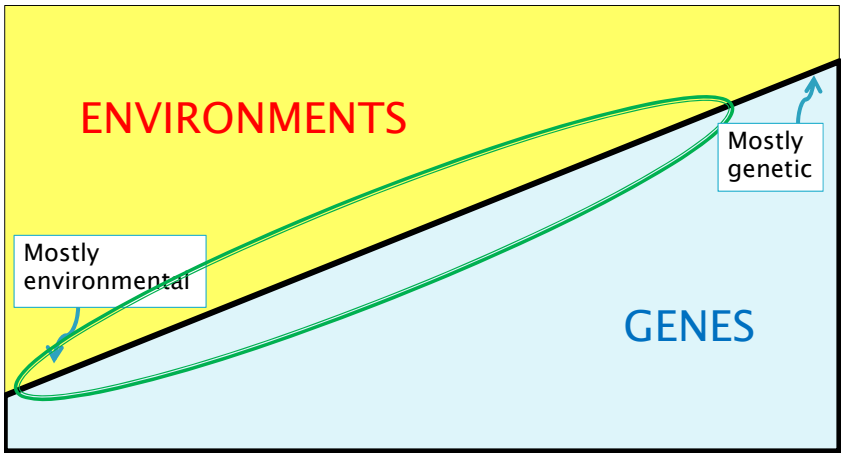
50 Years of Progress in Understanding the Causes of Three Common Birth Defects

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

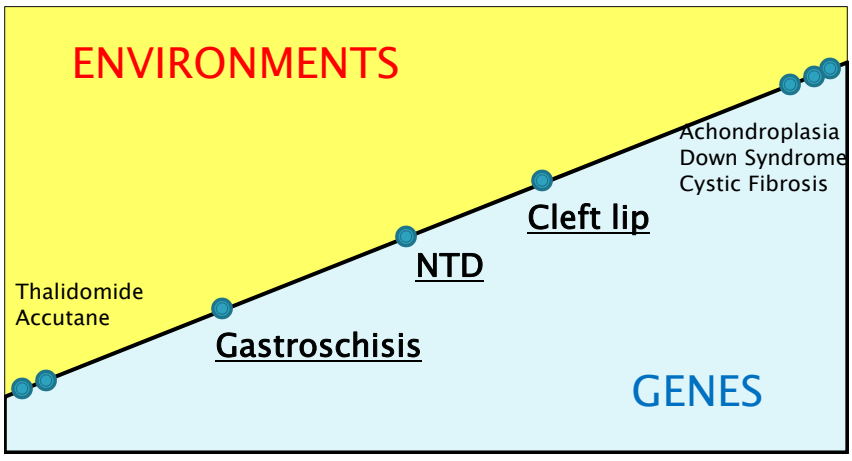
Genes/Environments: relative roles in etiology of birth defects



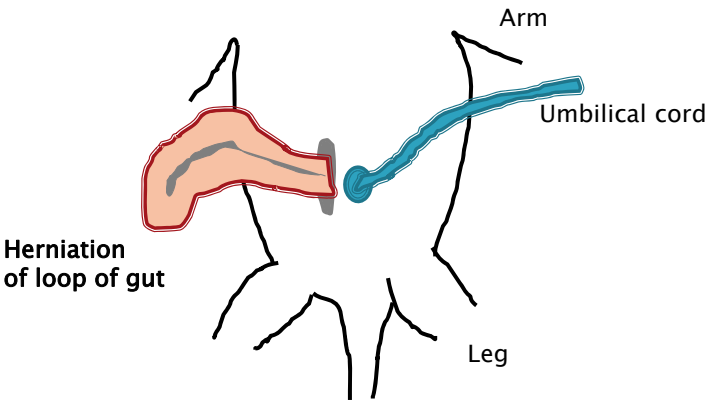
Genes/Environments: relative roles in etiology of birth defects



Genes/Environments



Gastroschisis



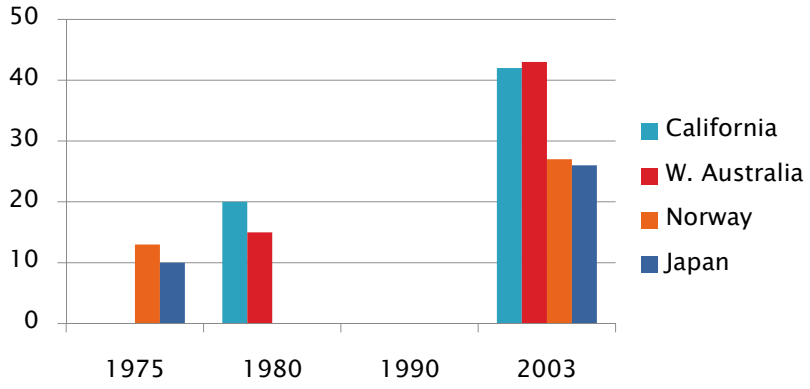
Adapted from: Brewer and Williams, 2004. *Bioessays 26:1307*

Gastroschisis ~50 years ago

Proc R Soc Med. 1967, 60(1):15-6.
Gastroschisis.
Young DC.

- No mention of genetics or environmental factors.
- Usually an isolated abnormality
- Incidence 1/17,000 births
- Long known. Cites article by Calder (1733)
- Distiguished from omphalocele

Gastroschisis rising frequency. Rate per 100,000 births.



Sources: Mastroiacovo et al 2006, BMJ 332:423.
Vu et al 2008, J Pediatr 152: 807.

Gastroschisis mouse models?

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

Aebp1 reference: Layne et al 2001, Molec Cellular Biol 21: 5256

Gastroschisis mouse models?

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?

Gastroschisis 2010

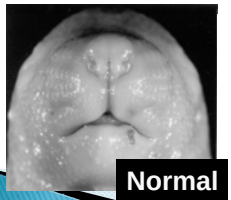
Marcia Feldkamp
1:00 today
Marriott Ballroom VI

"Gastroschisis: a journey to discover the pieces of the puzzle".

Cleft lip and palate

~1/1000 births. Populations differ.

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


Normal


Bilateral cleft


Left cleft

Cleft lip and palate: >50 years ago

Am J Surg. 1954 May;87(5):656-9.

The familial distribution of congenital clefts of the lip and palate; a preliminary report.

FRASER FC, BAXTER H.

Acta Genet Stat Med. 1955;5(4):358-69.

Thoughts on the etiology of clefts of the palate and lip.

FRASER FC.

Cleft lip and palate: 50 years ago

Science. 1960 Aug 12;132:420-1.

Influence of uterine site on occurrence of spontaneous cleft lip in mice.

TRASLER DG.

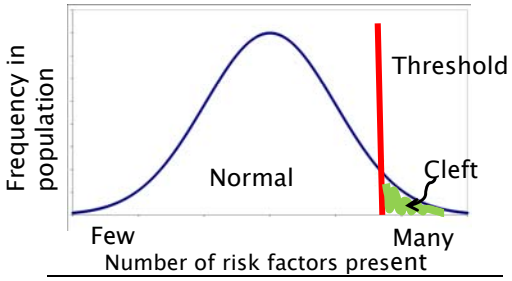
Teratology. 1968 Feb;1(1):33-49.

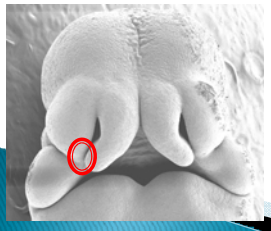
Pathogenesis of cleft lip and its relation to embryonic face shape in A-J and C57BL mice.

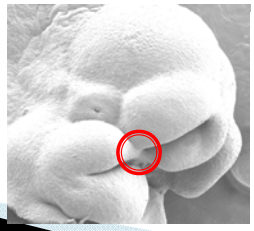
TRASLER DG.

CLP 1970's

Carter, Falconer, Fraser: multifactorial models

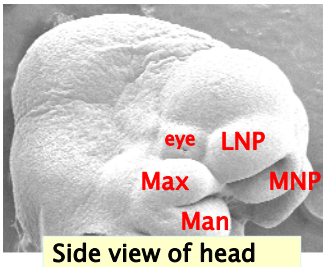




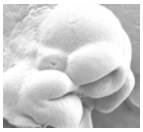


Three facial prominences of different origins have to grow together, meet and fuse in a narrow time widow.

Multifactorial CLP

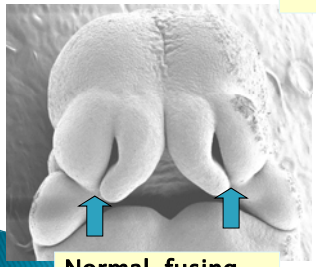


Side view of head

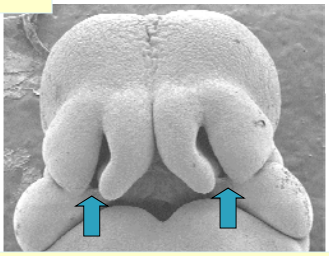


GD 11 embryos in the A/WySn mouse strain

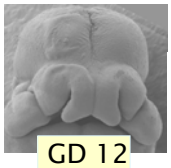
Front view



Normal, fusing



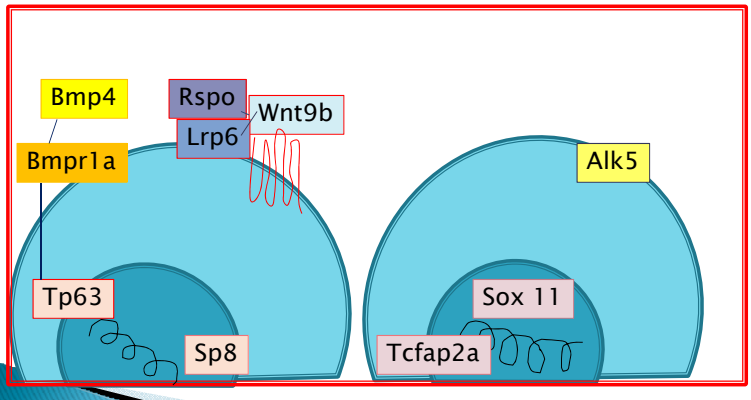
Bilateral cleft lip, not fusing



GD 12

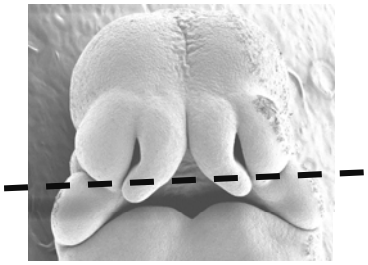
CLP 2010: Mouse CLP mutants

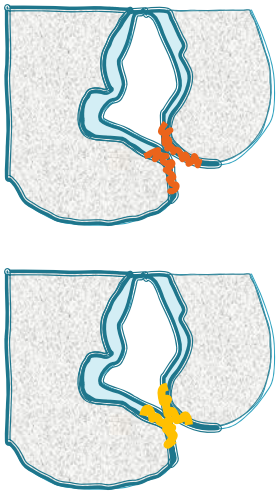
10 identified genes



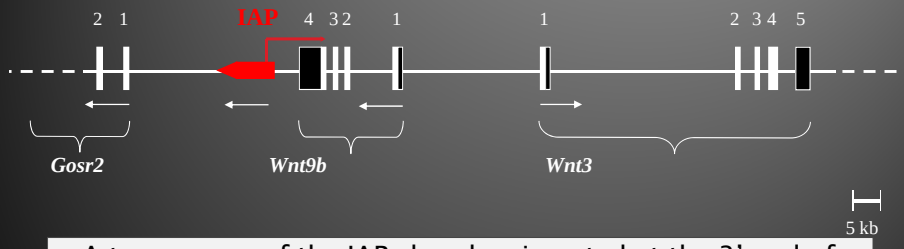
CLP genes: expression domains to be studied. Limited information.

GENES:
Bmp4
Bmpr1a
Sox11
Tcfap2a
Wnt9b
Lrp6
Tp63
Alk5
Rspo2





Mutation at Wnt9b gene



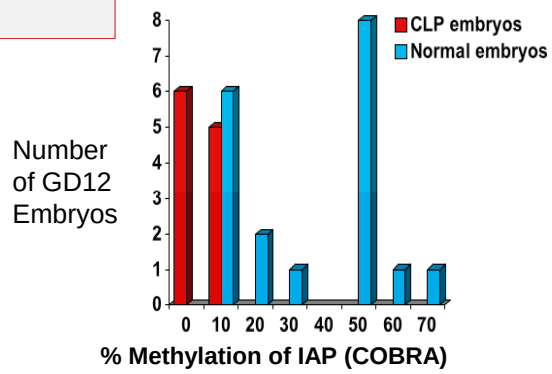
- A transposon of the IAP class has inserted at the 3' end of the *Wnt9b* gene.
- The IAP at this location is present all ALL A/WySn individuals.
- The IAP is not present at this location in other strains.

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.



CLP 2010: Examples of environmental factors

Periconceptional agent	Effect on CLP rate	Odds Ratio or Relative Risk	Example of References
Maternal heavy smoking	Increased	1.8	Honein et al., 2007
Maternal obesity (BMI)	Increased	1.2	Stothard et al., 2009
Maternal folic acid supplement	Decreased	0.5	Badovinac et al., 2007

CLP human genes 2010: examples

Positional cloning	Population	Candidate /linkage approach	Population
PVRL1	Venezuela	*IRF6*	Many (~10% of risk)
GWAS* on SNPs		*TGFB3*	China, Chile…
8q24, rs987525	European. Strong effect.	*MTHFR*	China…
17q22, rs 227731	European	*MSX1*	Various
10q25.3 rs7078160	European	*PTCH1*	Ireland …
MAFB, rs13041247	Asian	*FOXE1*	Various
ABCA4, rs560426	Asian		

CLP 2010 –still multifactorial

Case 1
genes

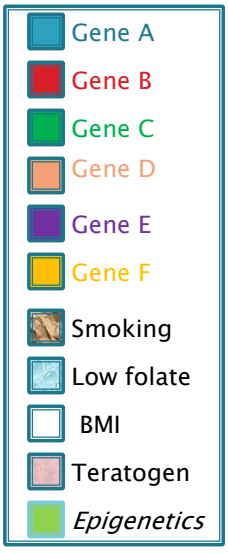
Case 2
genes +
environments

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

NTD 1960

- 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

NTD 1976-1989. Smithells and Folic acid supplementation (>20 papers)

1976 Smithells RW, Sheppard S, Schorah CJ. **Vitamin deficiencies and neural tube defects.** Arch Dis Child

1977 Smithells RW, Sheppard S, Schorah CJ. **Folates and the fetus.** Lancet

1980 Smithells RW, Sheppard S, Schorah CJ, Seller MJ,et al. **Possible prevention of neural-tube defects by periconceptional vitamin supplementation.** Lancet

1981 Smithells RW, Sheppard S, Schorah CJ, Seller MJ,et al. . **Apparent prevention of neural tube defects by periconceptional vitamin supplementation.** Arch Dis Child

1983 Schorah CJ, Wild J, Hartley R, Sheppard S, Smithells RW. **The effect of periconceptional supplementation on blood vitamin concentrations in women at recurrence risk for neural tube defect.** Br J Nutr

NTD 1976-1989. Smithells and Folic acid supplementation (>20 papers)

To settle the debate...

1985 Smithells RW, Sheppard S, Wild J, Schorah CJ, Fielding DW, Seller MJ, et al. Neural-tube defects and vitamins: the need for a randomized clinical trial. Br J Obstet Gynaecol

NTD: The MRC Multicenter Study on prevention of recurrence (Lancet 338:131-137, 1991)

Group *	NTD / informative pregnancies	NTD
Folic Acid (4 mg)	2/298	1%
Folic Acid (4 mg) plus vitamins	4/295	
Neither	13/300	3.5%
Vitamins	8/302	
	TOTAL = 1195 pregs	

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

*Periconceptional until week 12

NTD: The Hungarian randomized study of prevention of occurrence (N Engl J Med 327:1832-1835, 1992)

Group*	NTD/informative pregnancies
Folic Acid (0.8 mg), vitamins, minerals	0/2104
Minerals	6/2052

P = 0.03

•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.

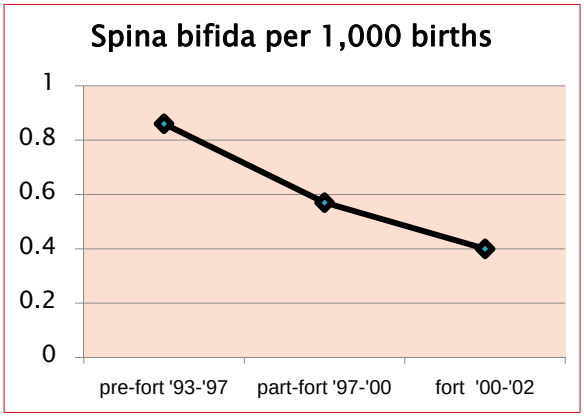
* Preconceptional until 3rd month

NTD: Folic acid fortification of flour

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

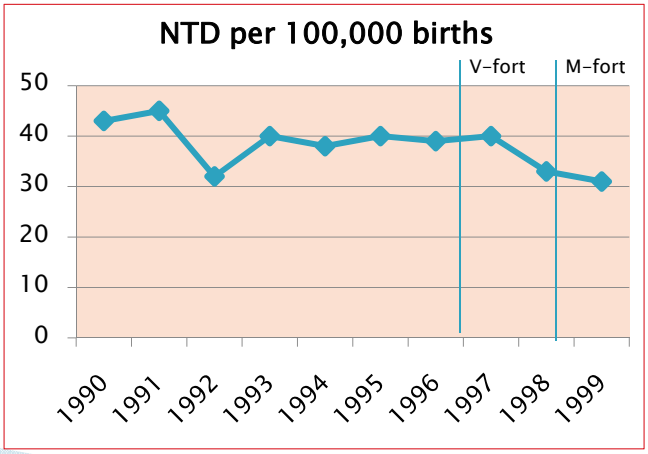
Canadian study.
1,900,000 births.
P < 0.0001

DeWals et al (2008)
BDRA 82:622-626.



NTD: USA folic acid fortification

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



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.

NTD Genetics 2010

- 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.

NTD Genetics 2010

- 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.

NTD Genetics 2010

- 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

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

Harris and Juriloff, 2007 & 2010

Mouse mutants with folate pathway gene mutations that survive to neural tube closure mostly do not have NTDs

Genes		Genes	
Cbs	normal neural tube	*Mthfr*	normal neural tube
Folr2	normal neural tube	*Mtrr hypomorph*	normal neural tube
Mthfd1	normal neural tube	*Shmt1*	normal neural tube
Mthfd2	normal neural tube	*Slc46a1 (PCFT)*	normal neural tube
Folr1 + folinic acid	NTD	*RFC1* + folic acid	NTD

Harris and Juriloff, 2007 & 2010

Mouse NTD mutants and folic acid

Mutants that respond to folic acid	Function of gene
Cart1	Transcription
Cited2	Transcription coactivator
Lrp6 (Cd)	Wnt signaling
Gcn5	Chromatin structure
Pax3 (Sp)	Transcription
Folr1	Folate receptor

Mutants that do not respond to folic acid	Other effective agents
Axd	methionine
Grhl3 (ct)	Inositol, retinoic acid
Fkbp8	--
Map3k4	--
Nog	--

Harris and Juriloff, 2007 & 2010

NTD Epigenetics 2010

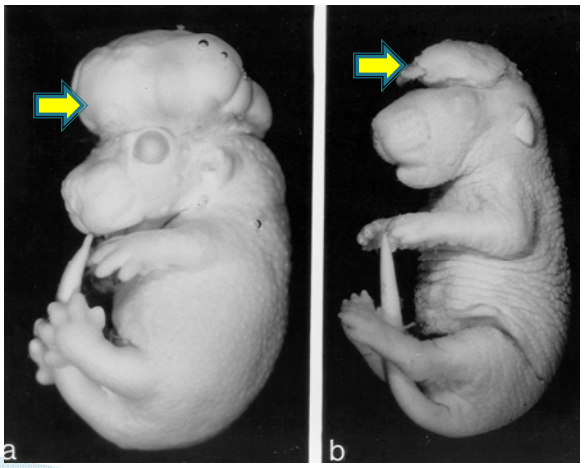
- Recent report from China that NTD cases have hypomethylated DNA.

(Wang, Am J Clin Nutr 2010, 91:1359).

Neural Tube Development

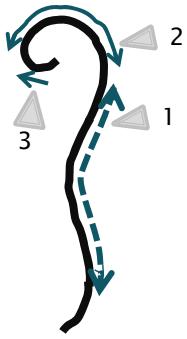
Most of what we know is based on mouse.

Exencephaly in Embryo and fetus

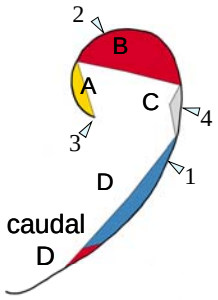


Neural fold elevation zones and fusion initiation sites.

Sites of common NTDs relate to elevation zones.

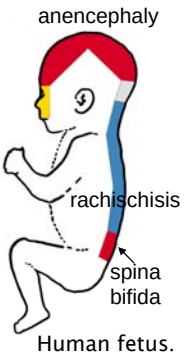


Human



Day 9 mouse embryo.

Mouse fetus.

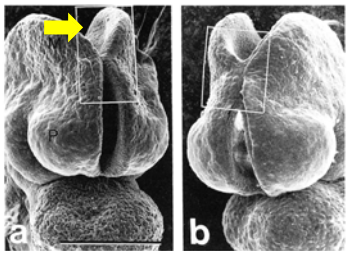


Human fetus.

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

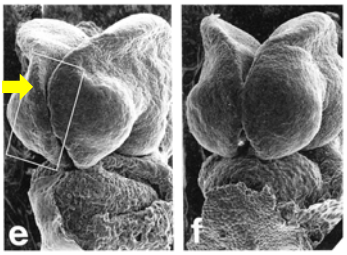
Neural Tube 2010

Strain 1



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

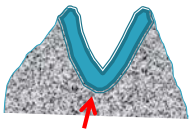
Strain 2



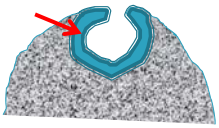
These normal variants may affect liability to teratogens that cause NTD
Or
Amount of NTD in the presence of a mutation.

Source: Juriloff et al., Teratology, 1991

Neural tube closure 2010

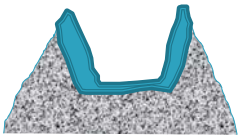


Median hinge point (MHP)
Anterior spine

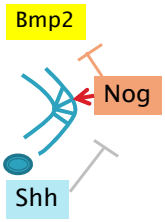


Lateral hinge point (LHP).
Caudal spine and the head.
This is what seems to fail in exencephaly and spina bifida

Ref: Copp & Greene.
J Pathol 2009



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



CLP and NTD 2010 –multifactorial

Case 1
genes

Case 2
genes +
environments

Case 3
other genes +
other environments

Case 4
genes +
teratogen

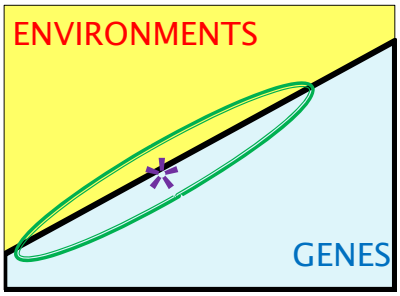
Case 5
genes +
environment +
epigenetics

Case 6
environment +
teratogen +
epigenetics

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?

END