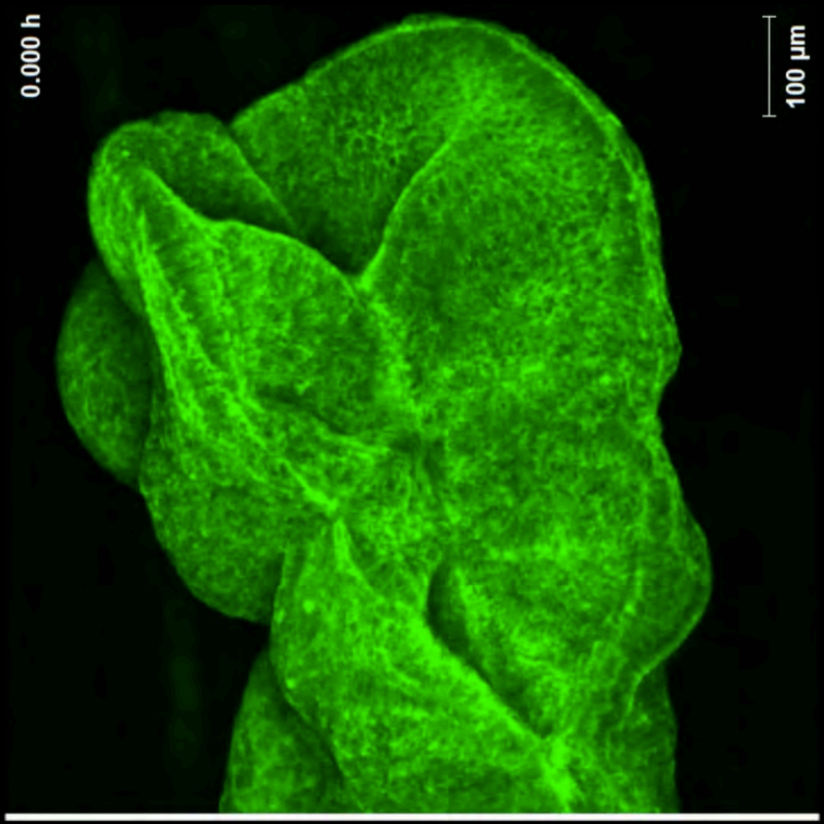
II. Immunology and Inflammation

4:45 PM

Too Much of a Good Thing? Considering Gene-Environment Interactions in Health and Disease

Lee Niswander, Ph.D. – Chair of Molecular, Cellular, and Developmental Biology, University of Colorado Boulder

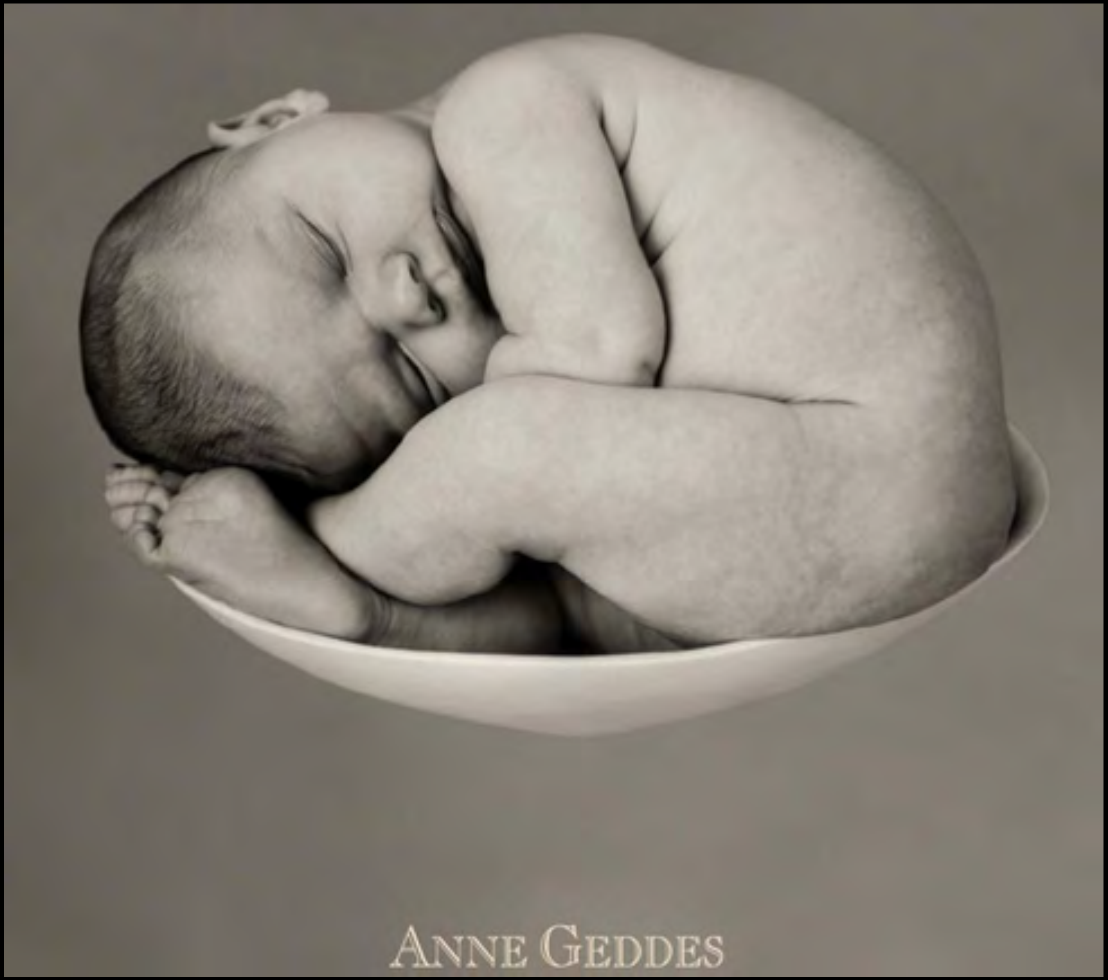
Too much of a good thing?
Considering gene-environment interactions in health and disease.



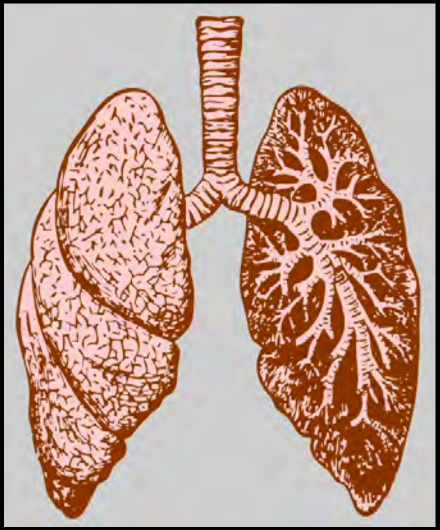
Lee Niswander, Ph.D.
Univ of Colorado Boulder
Univ of Colorado Medical Campus
Children's Hospital Colorado

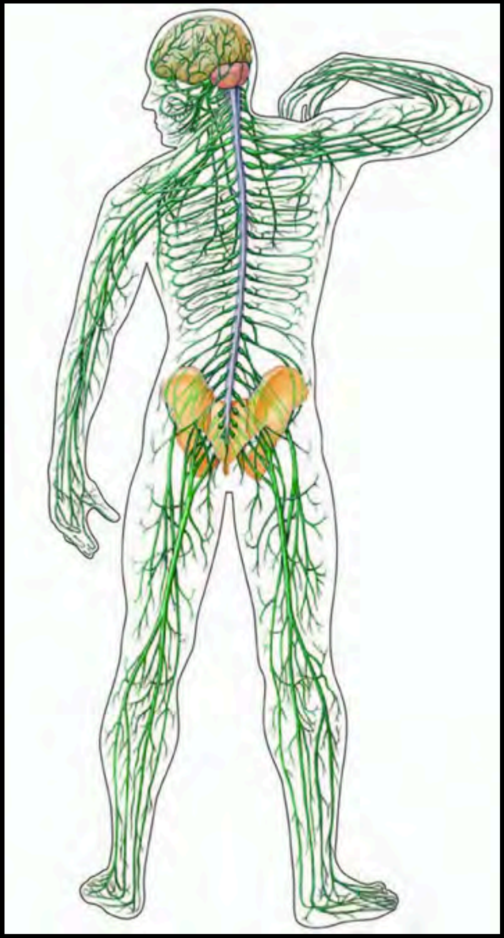
Developmental Biology to understand the causes of birth defects

?



Developmental Origins of Health and Disease





Neural Tube Defects (spinal cord/brain)

NTDs = Failure of Neural Tube Closure
~1:1000 births worldwide

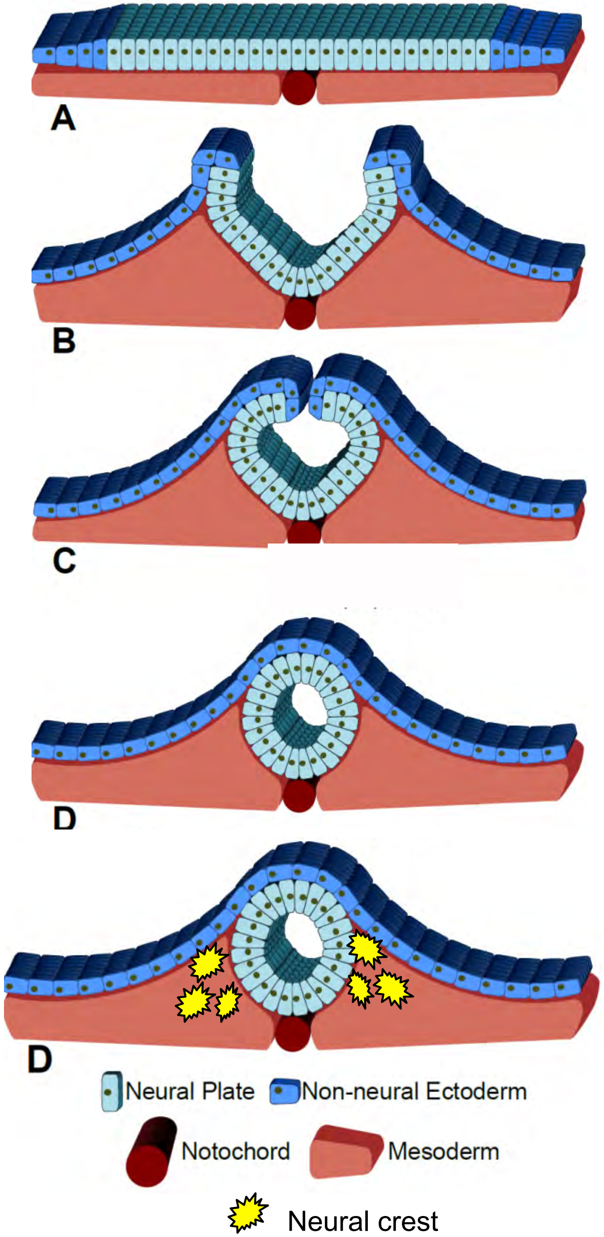
Spina Bifida: failure of lower neural tube to close

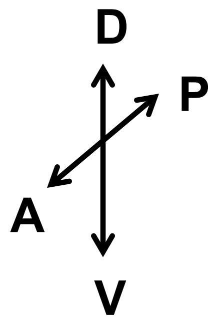
- Increased mortality
- Life-long morbidities
  - Neurologic/Neurosurgical
  - Urologic
  - Orthopedic
  - Psychological

Anencephaly: failure of cranial neural tube to close

- Lethal

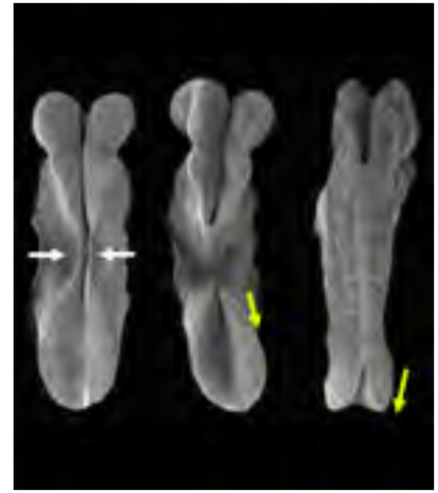
Early Brain and Spinal Cord Development: Neural Tube Closure





mouse

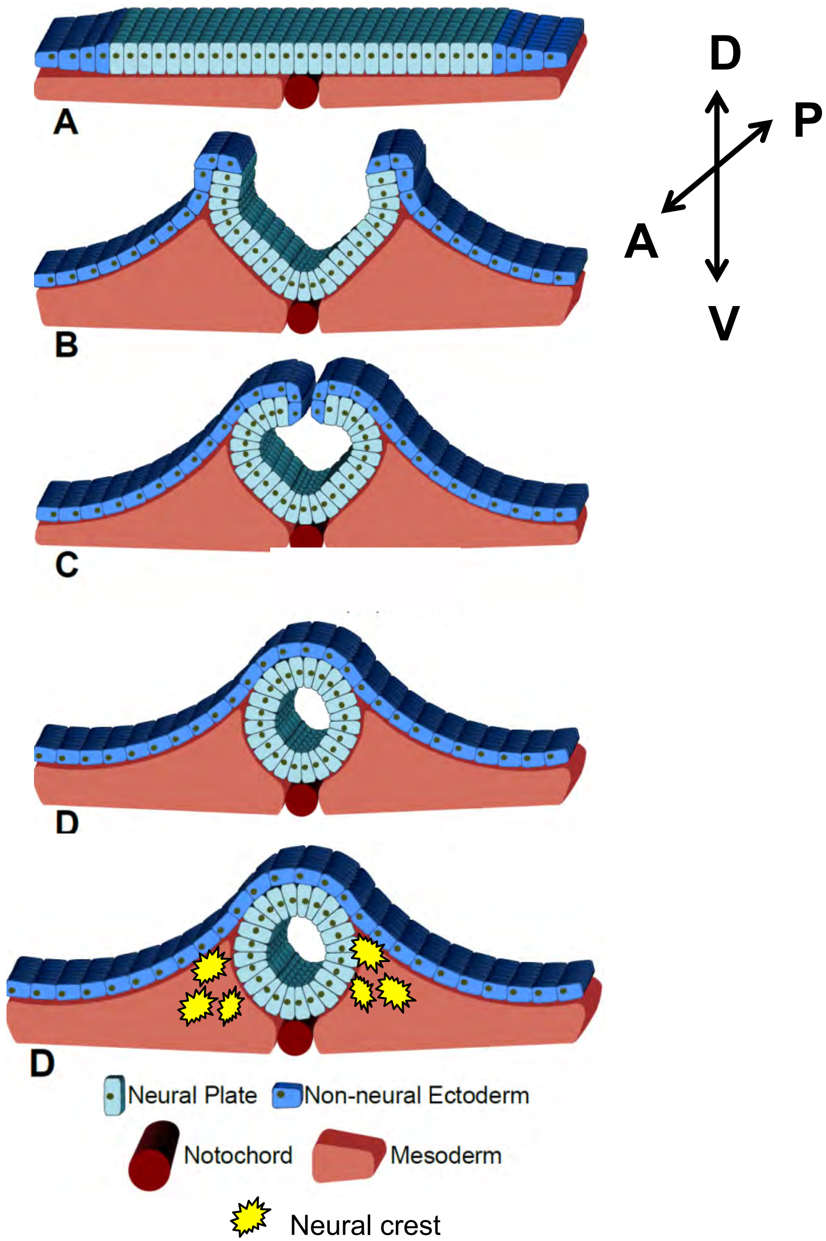
E8.25 – 10.25



human

Week 3-4

Early Brain and Spinal Cord Development: Neural Tube Closure



Coordinate:

- Patterning
- Growth
- Differentiation
- Cell death
- Cell movements
- Cell architecture
- Tissue interactions
- Physical forces

Early brain and spinal cord formation

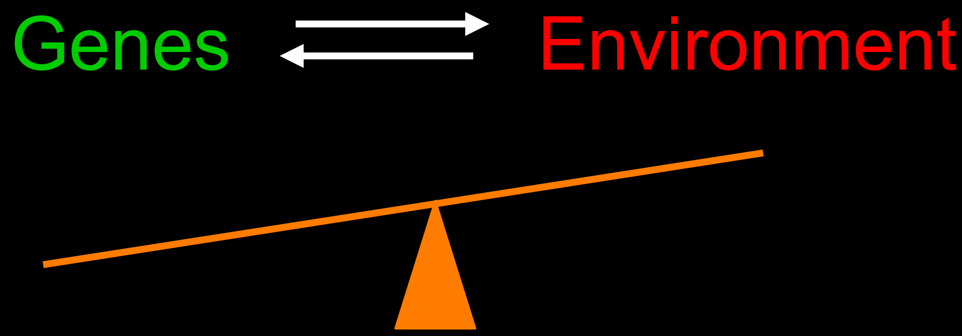
Week 3-4: Before woman knows she is pregnant

Timing of closure is critical

Best treatment for NTD is to close the defect…surgery (postnatal or fetal)

Prevention

Strategies for Prevention: Gene-Environment Interactions

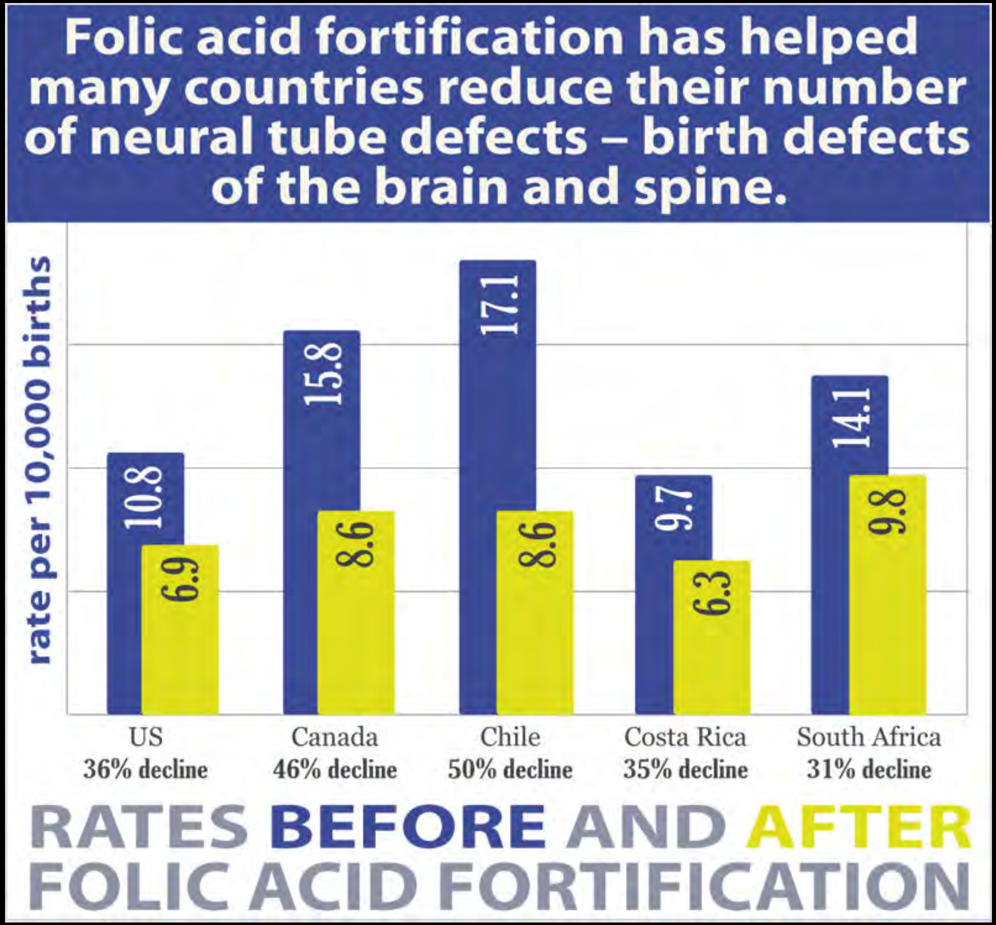


Environmental risk factors for NTDs

- Teratogens:
  - valproic acid, carbamazepine, trimethoprim
- Maternal obesity
- Maternal diabetes/hyperglycemia
- Maternal hyperthermia
- Maternal nutrient deficiencies:
  - **Folic acid, zinc, iron**

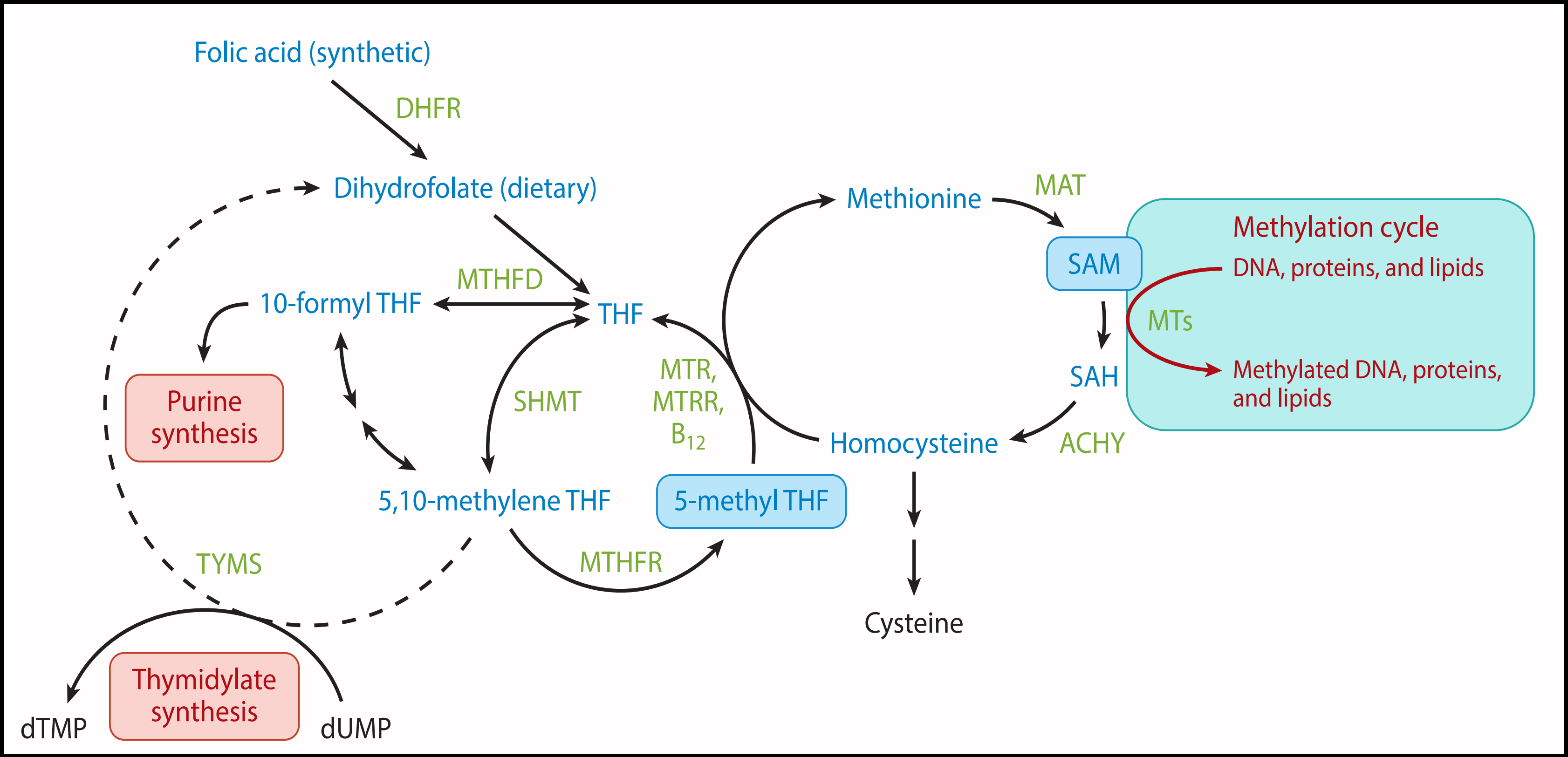
Benefits of Folic Acid Fortification

- Folic acid studies began in 1960s, landmark random clinical trials in 1990s
- Mandatory U.S. grain supply fortification started January 1998
- ~35% decrease in NTDs in the U.S.



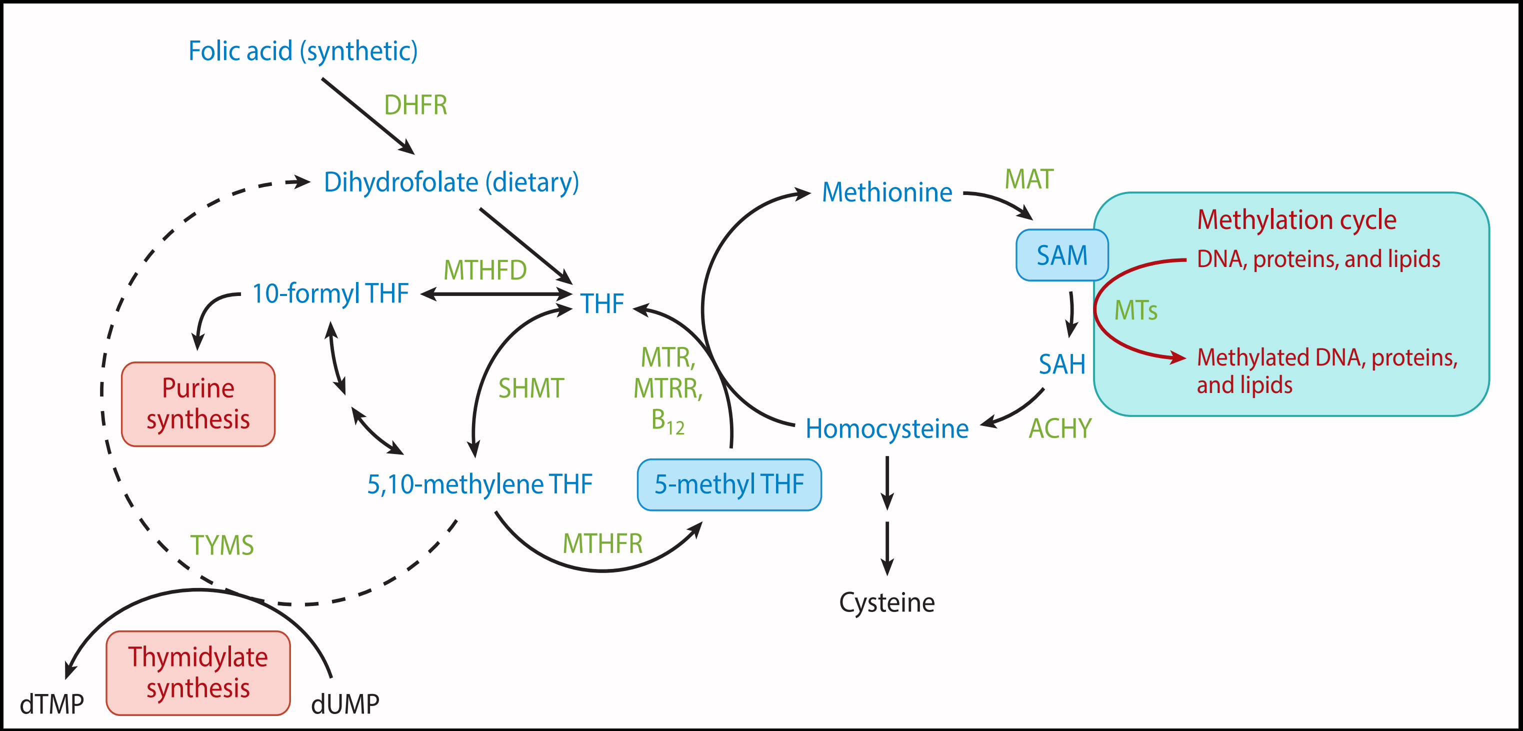
- How does folic acid prevent NTDs?
- Which mutations/gene pathways benefit from folic acid?

Folic acid is needed for the production of purines, thymidylate, and SAM



Is there a strong correlation between folate pathway mutants and NTDs?
NO

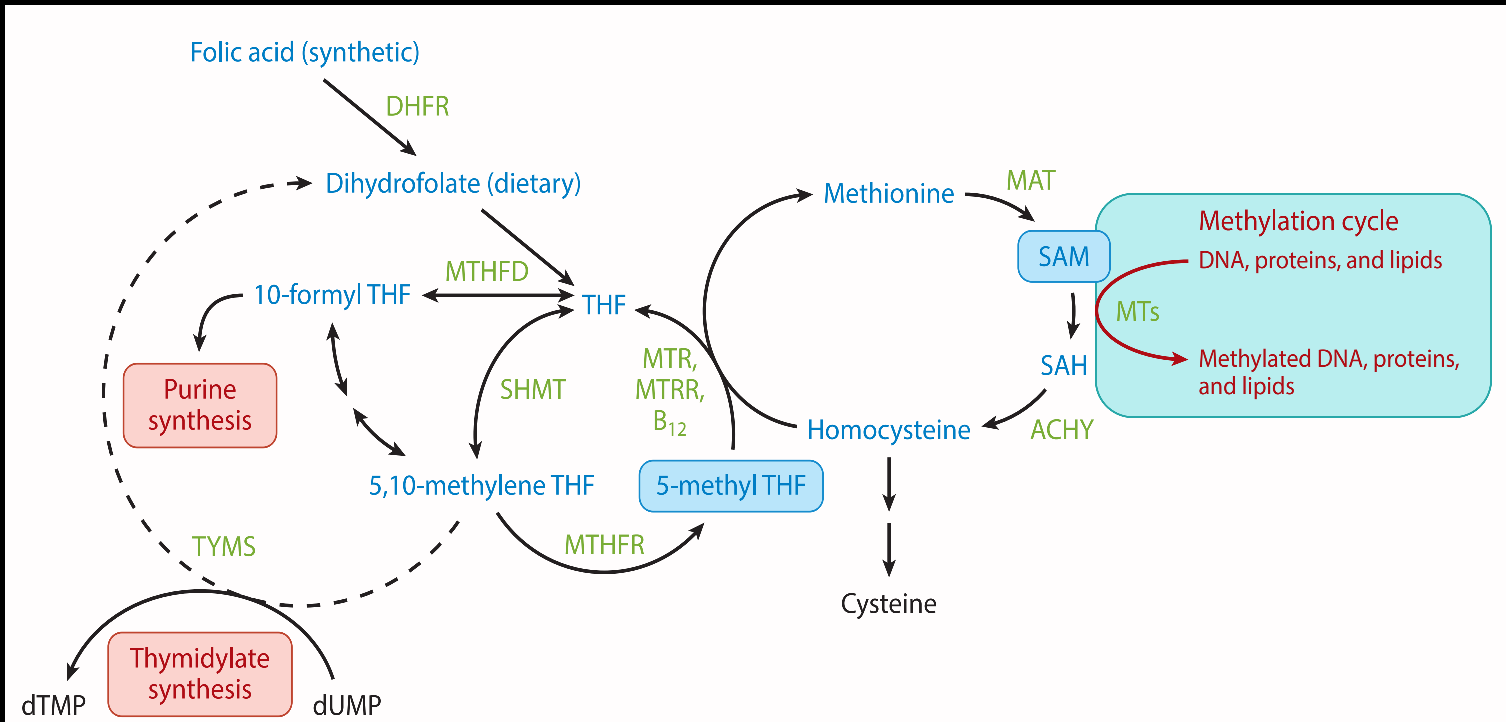
How does folic acid act during neural tube closure?



Folate deficient

Cell proliferation and survival

How does folic acid act during neural tube closure?



Folate replete
Long-term fortification & supplementation

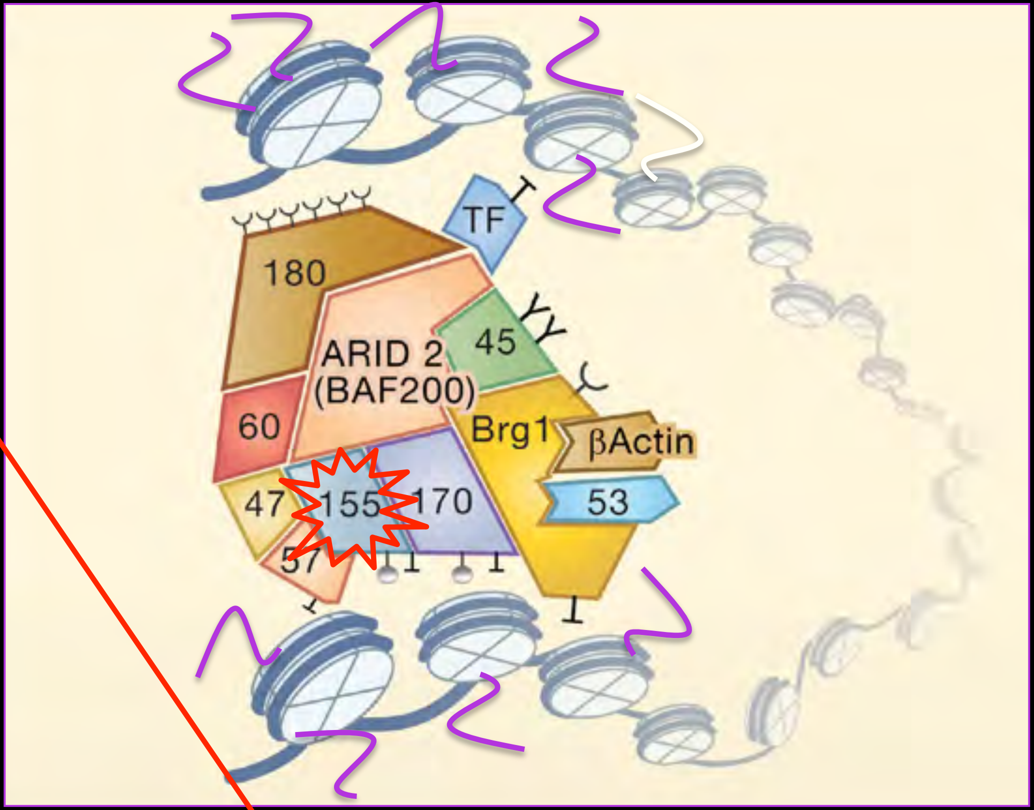
Folate deficient

Methylation changes?
Epigenetic regulation?

Cell proliferation and survival

Chromatin modifying enzymes and neural tube defects

Protein	Function
Baf155	Chromatin remodeling
Baf47	Chromatin remodeling
Brg1	ATPase of chromatin remodeling
Nap 1/2	Histone Chaperone- nucleosome assembly
CBP	HAT/Transcriptional activation
P300	HAT/Transcriptional activation
GCN 5	Histone Acetyltransferase
HDAC4	Histone Deacetylase
Sirt1	Deacetylase
Brd2	Histone Modification

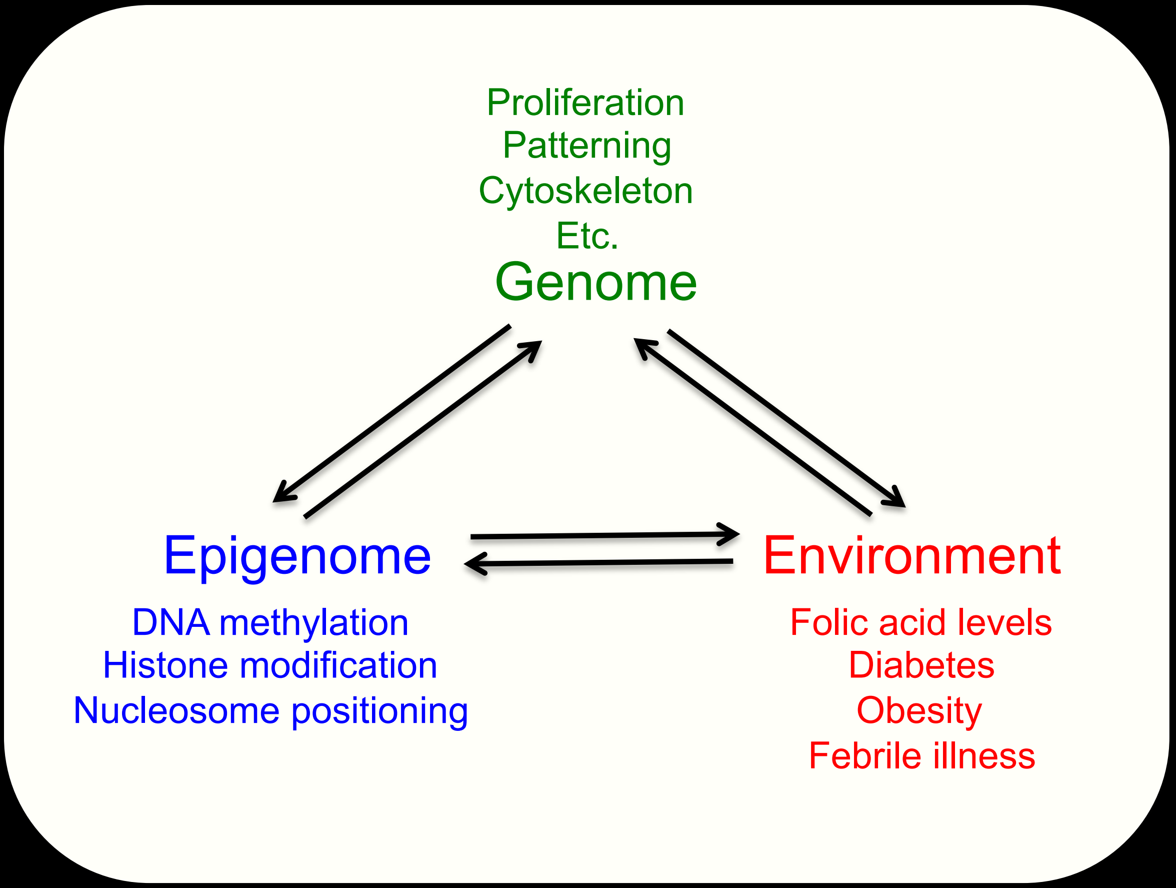


Crabtree et al 2010

Copp and Greene, J Pathology 2009
Harris and Juriloff, Birth Defects Res 2010

Valproic acid: histone deacetylase inhibitor

How does folic acid act during neural tube closure?

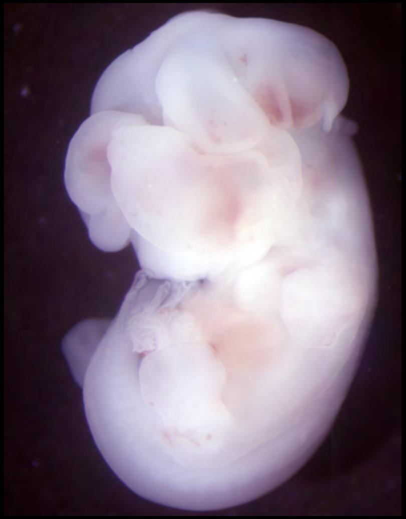


Genetics of neural tube closure using mouse models

- What are the genes involved?

Mouse studies
Genetic Screens

wildtype



Exencephaly
Cranial NTD

Spina bifida
Caudal NTD

- How do these genes work?

- What goes wrong to cause neural tube defects?

Niswander lab contributions to understanding neural tube closure

Patterning/cilia

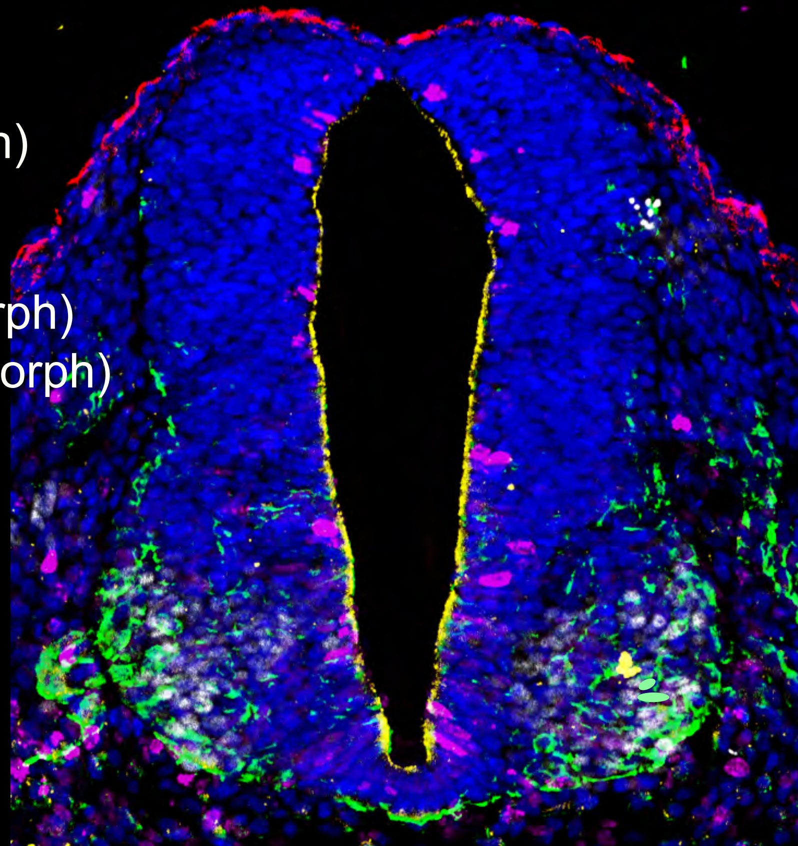
Ift88 (null & hypomorph)
Ift52
3poly
C2cd3 (null & hypomorph)
Inturned (null & hypomorph)
Fuzzy
Mks1
Ccdc40
PigN
Pgap1
Snx3
Tmem132a

Migration

Phactr4

Tissue Interactions

Hectd1
Baf155



Cell adhesion

Grhl2
Frem2
AP2α
Ryr1
p38IP (null & hypomorph)

Cell architecture

Shroom3
Grhl3

Proliferation
Differentiation

mLin41
Phactr4
Wdr62
Gcn5

Cell specification

Fpn1
Pax3
Zic2

Environmental Factors

Folic acid
Iron
Zinc

Impacting child health

Mouse models of NTDs

Human NTD genomic information

Animal models: causative role & genetic interplay

Environmental Impact

Fetal surgery
Biomaterials, Stem Cells, NTD modeling

Mouse NTD models to uncover the genetics of responsiveness to folic acid

To better reflect current US folic acid intake:

- **Moderate and enriched folic acid diets that correlate with pre- and post-fortification diets**
- **Long-term diet over multiple generations**

What pathways or cellular functions are responsive?

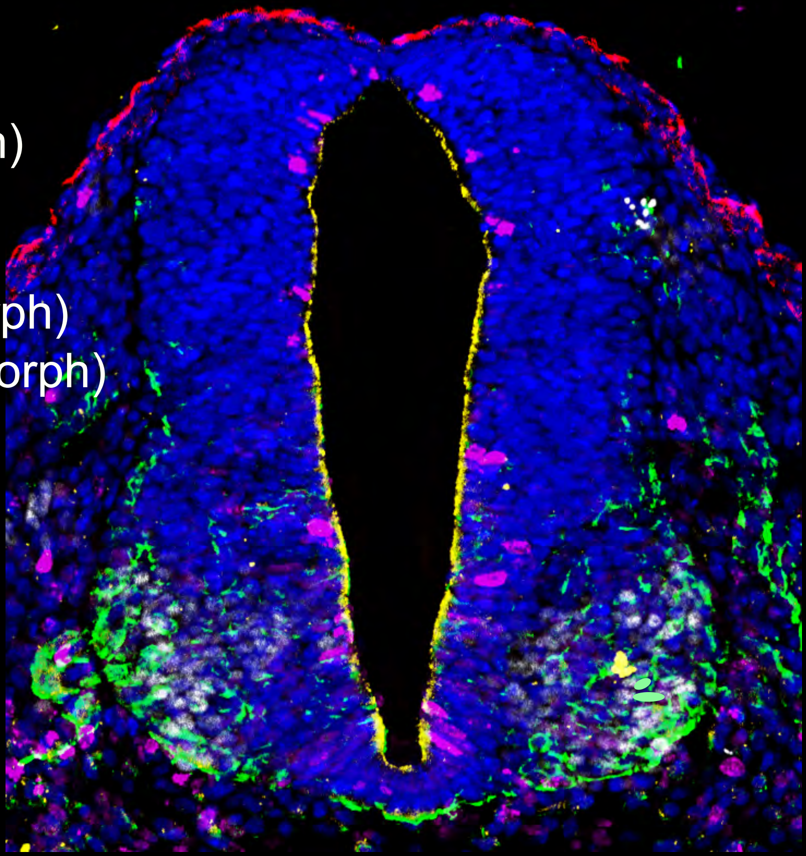
Additional therapies for folic acid non-responsive NTDs?

Mouse NTD models to uncover the genetics of responsiveness to folic acid

Patterning/cilia
Ift88 (null & hypomorph)
Ift52
3poly
C2cd3 (null & hypomorph)
Inturned (null & hypomorph)
Fuzzy
Mks1
Ccdc40
PigN
Pgap1
Snx3
Tmem132a

Migration
Phactr4

Tissue Interactions
Hectd1
Baf155



Environmental Factors
Folic acid
Iron
Zinc

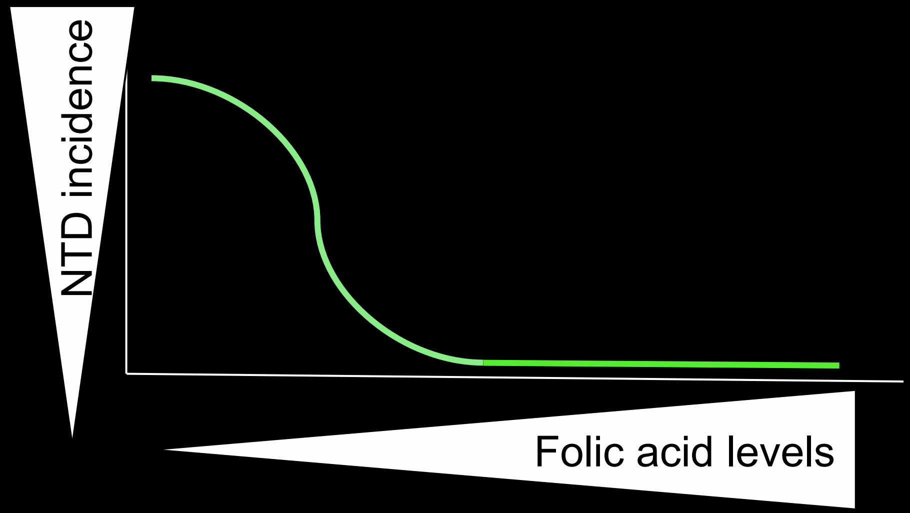
Cell adhesion
Grhl2
Frem2
AP2α
Ryr1
p38IP (null & hypomorph)

Cell architecture
Shroom3
Grhl3

Proliferation Differentiation
mLin41
Phactr4
Wdr62
Gcn5

Cell specification
Fpn1
Pax3
Zic2

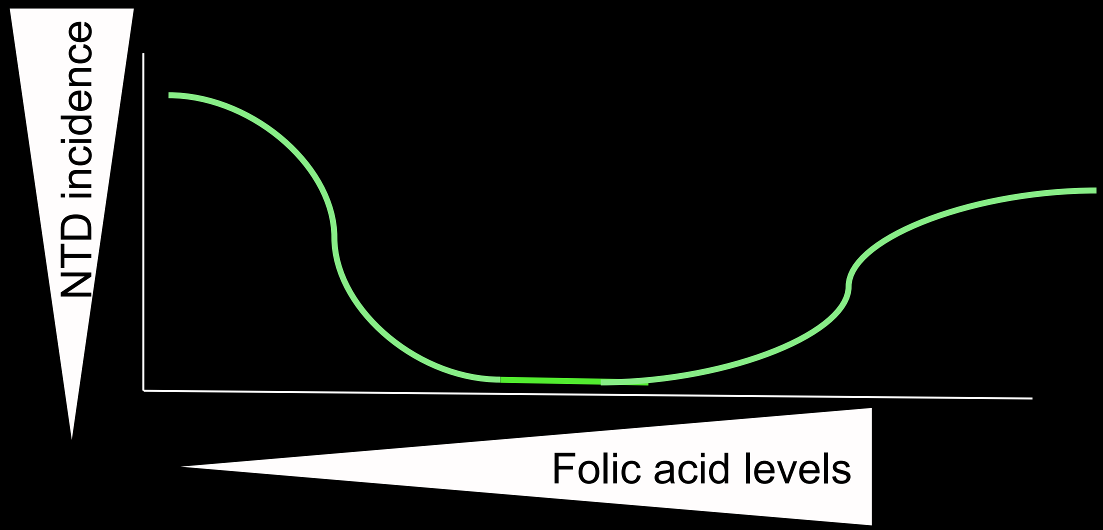
Low folic acid levels can increase the risk for NTD



Is NTD prevention always due to rescue?

No, early embryonic lethality

Low folic acid levels can increase the risk for NTD

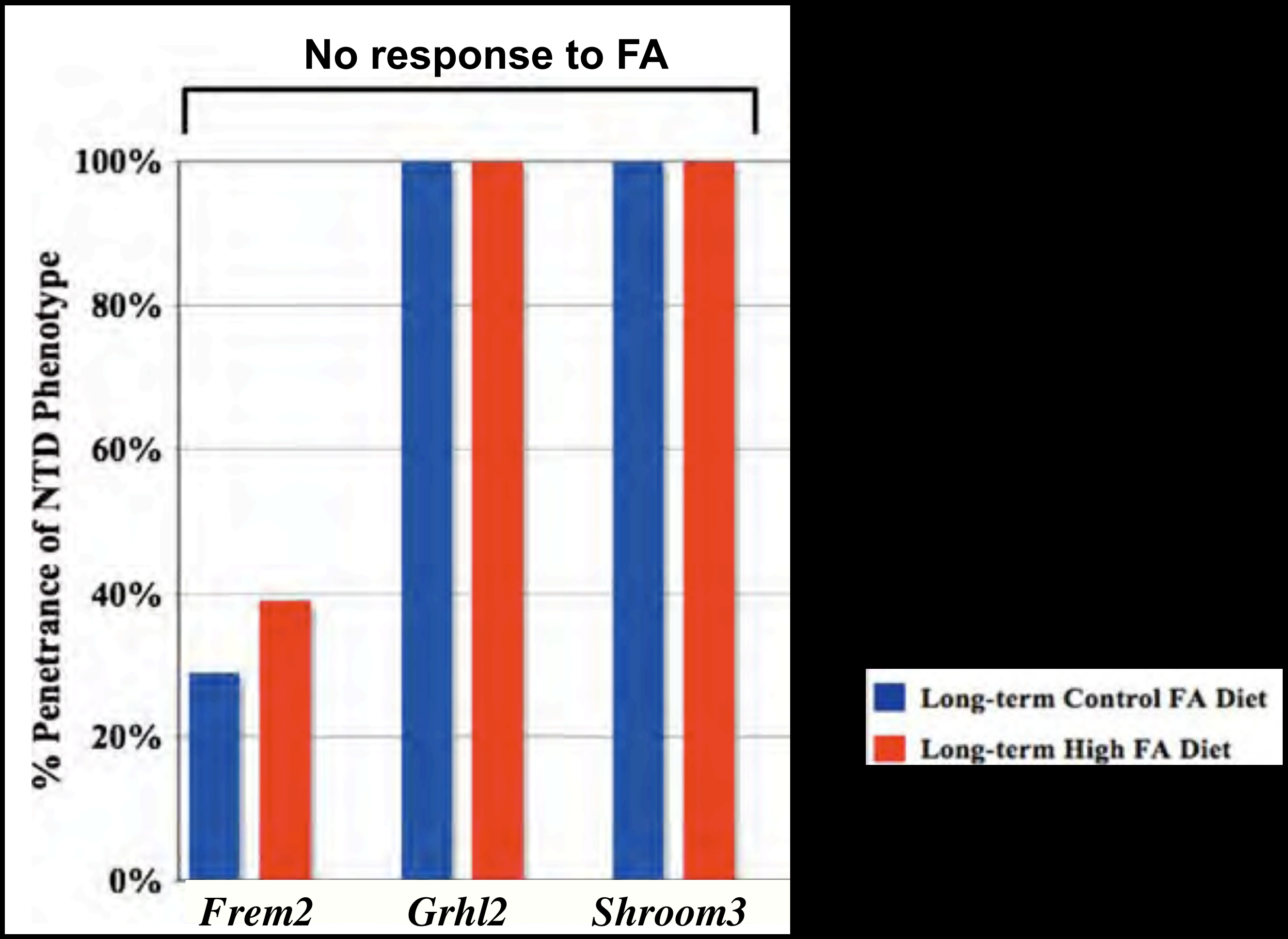


Balance? Might there be a dose that exceeds a beneficial level in the context of genetic mutation?

Unexpected increased NTD risk on enriched folate diet

Can length of exposure affect the outcome?
Yes,…. epigenetic?

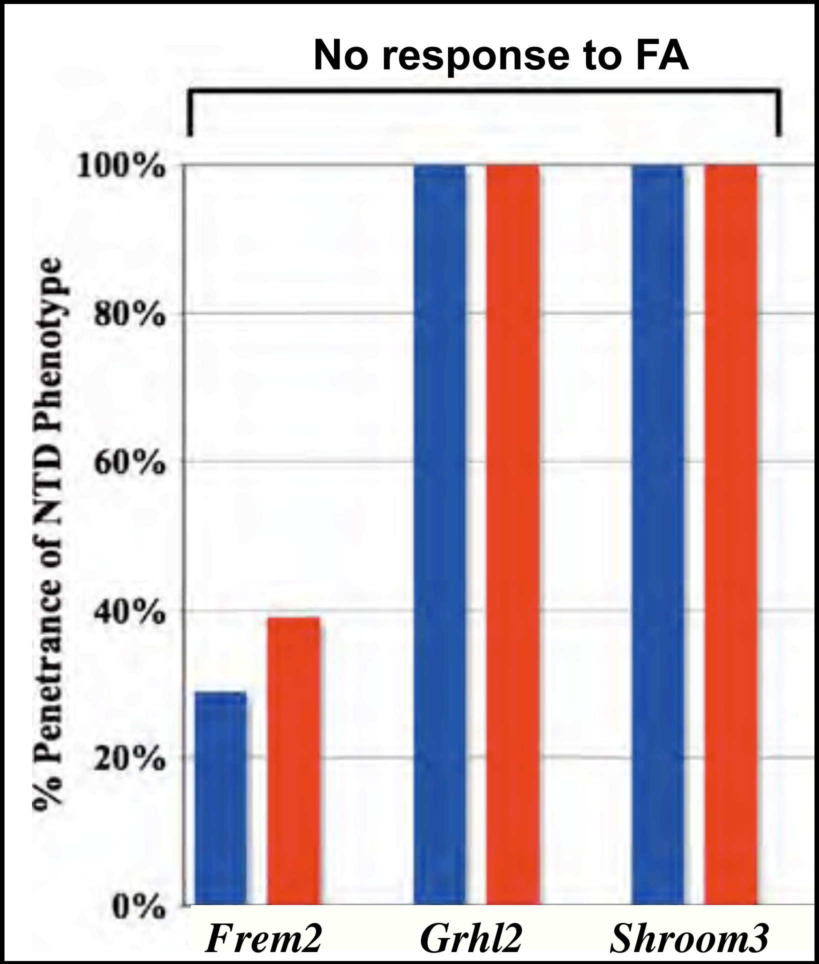
Folic Acid Responsive in Mouse Models

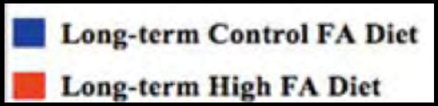


11 cases
Non-responsive

Human Molecular Genetics, 2011

Folic Acid Response in Mouse Models



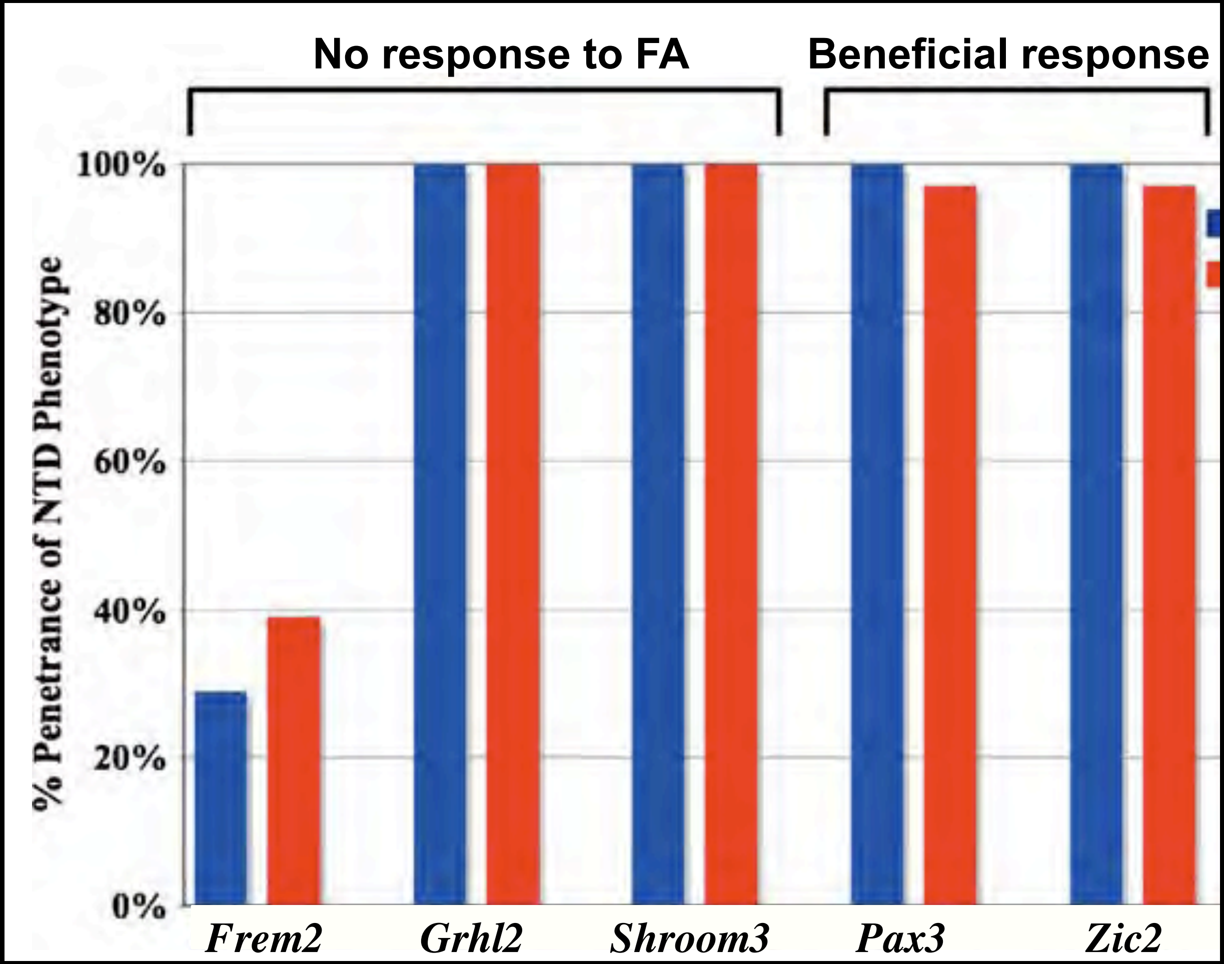


Shroom3
short-term FA beneficial

11 cases
Non-responsive

Human Molecular Genetics, 2011

Folic Acid Response in Mouse Models

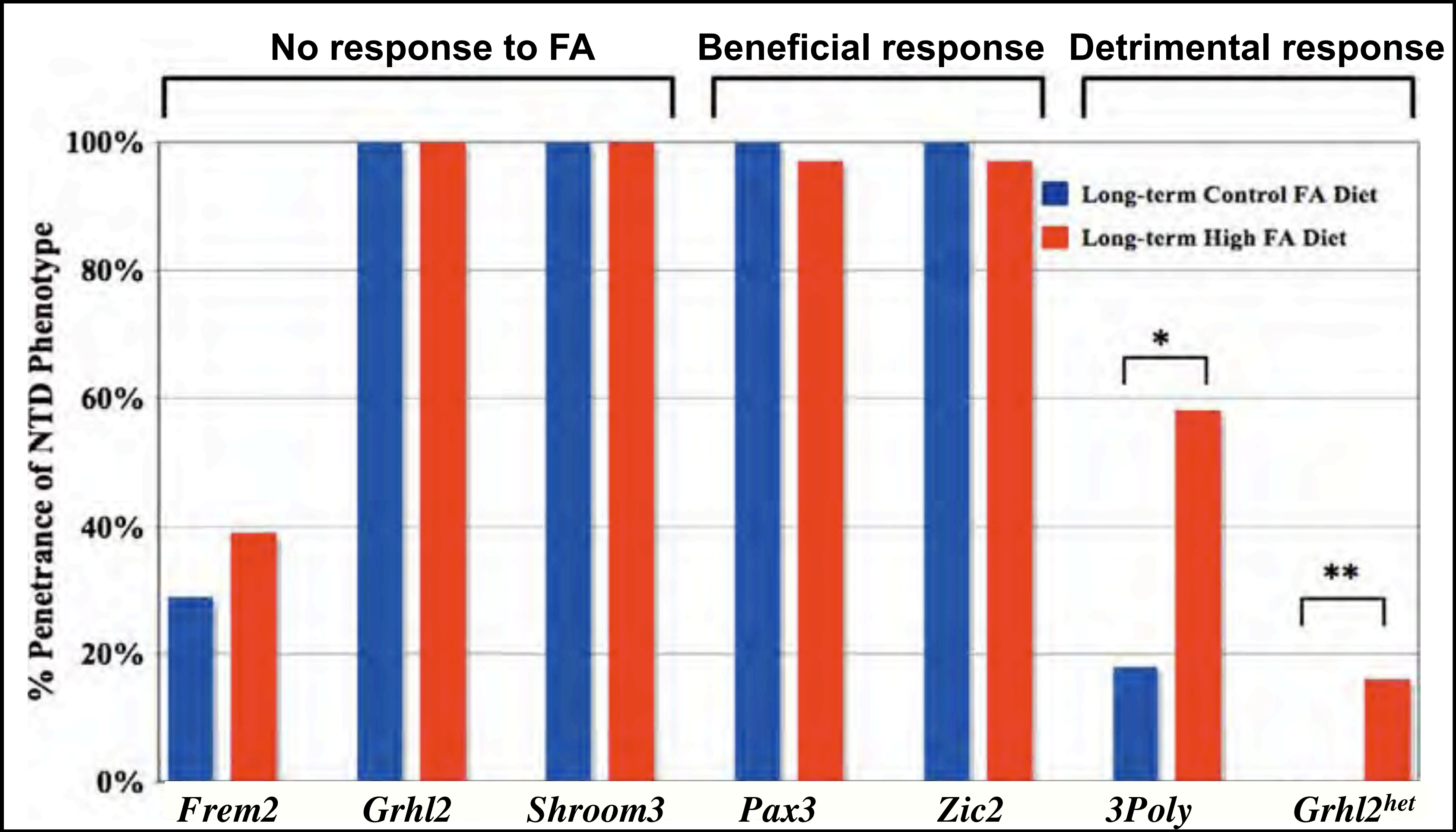


11 cases
Non-responsive

8 cases
Beneficial response

Human Molecular Genetics, 2011

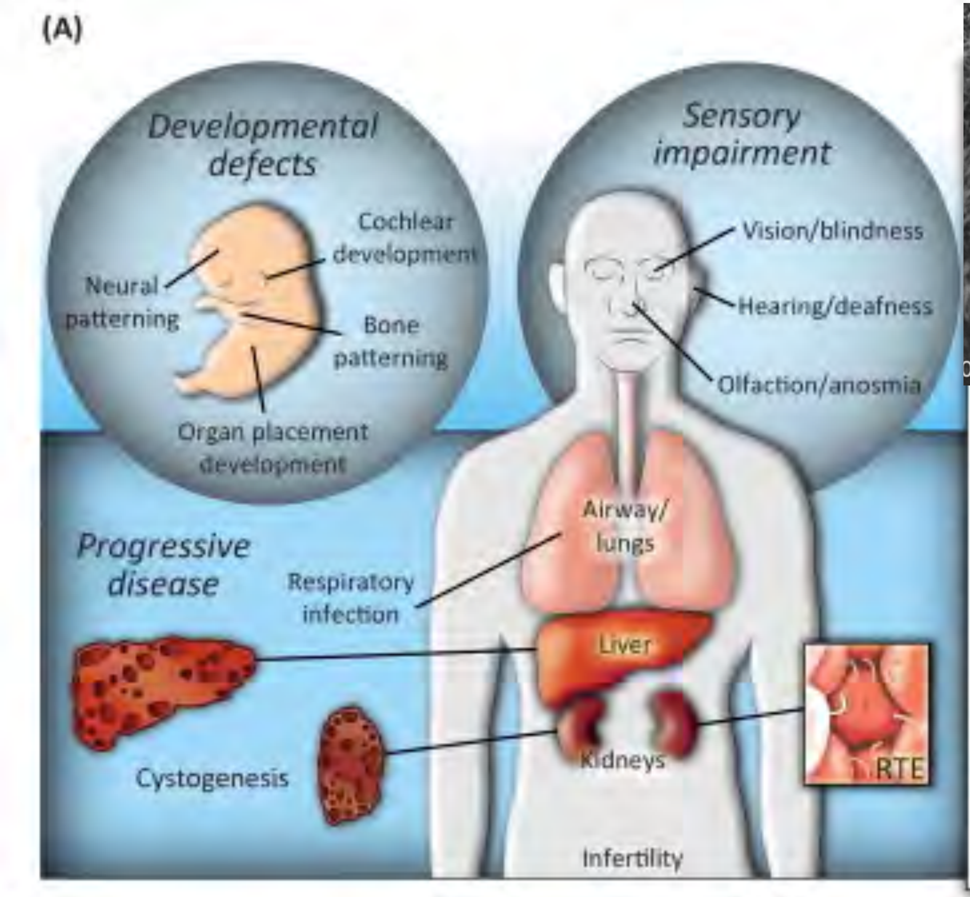
Folic Acid Response in Mouse Models

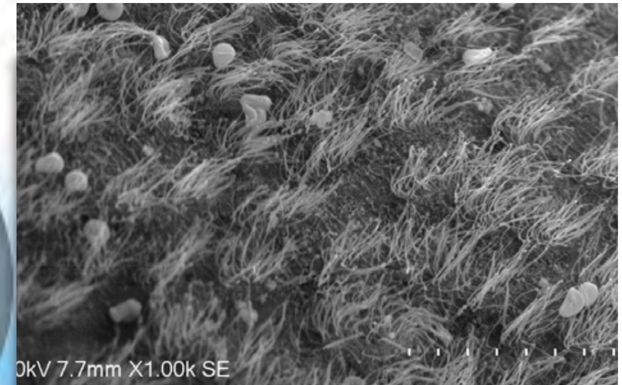


11 cases
Non-responsive

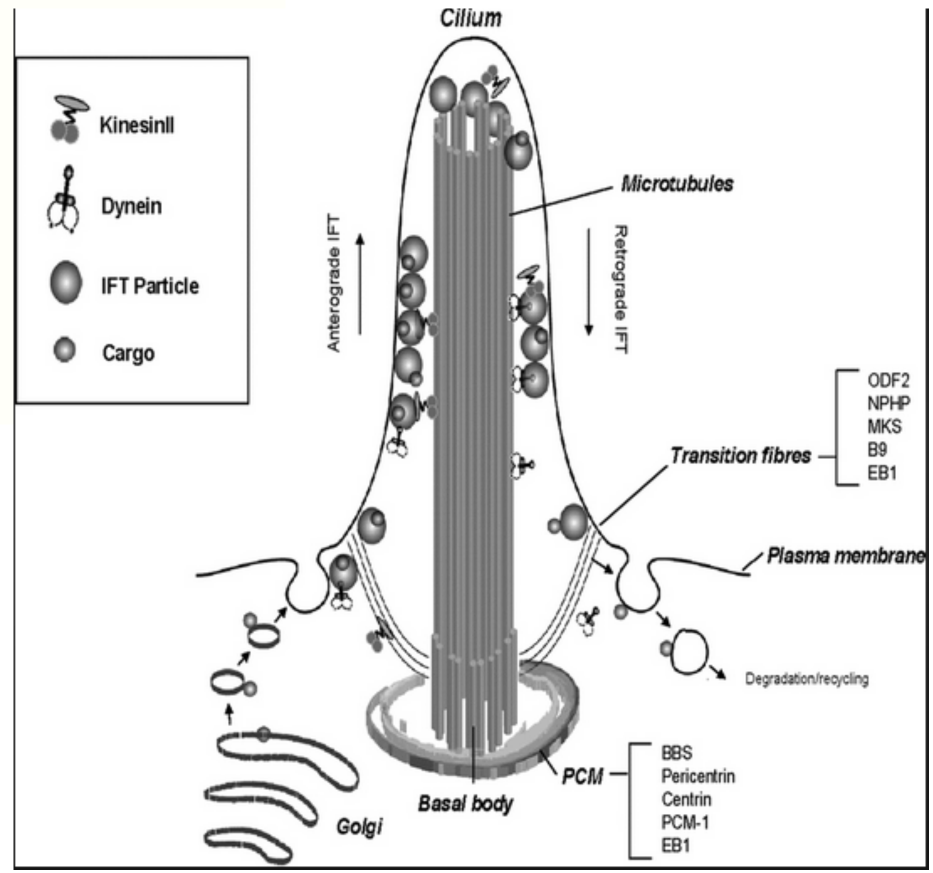
8 cases
Beneficial response

3 cases
Detrimental response
Link to cilia?

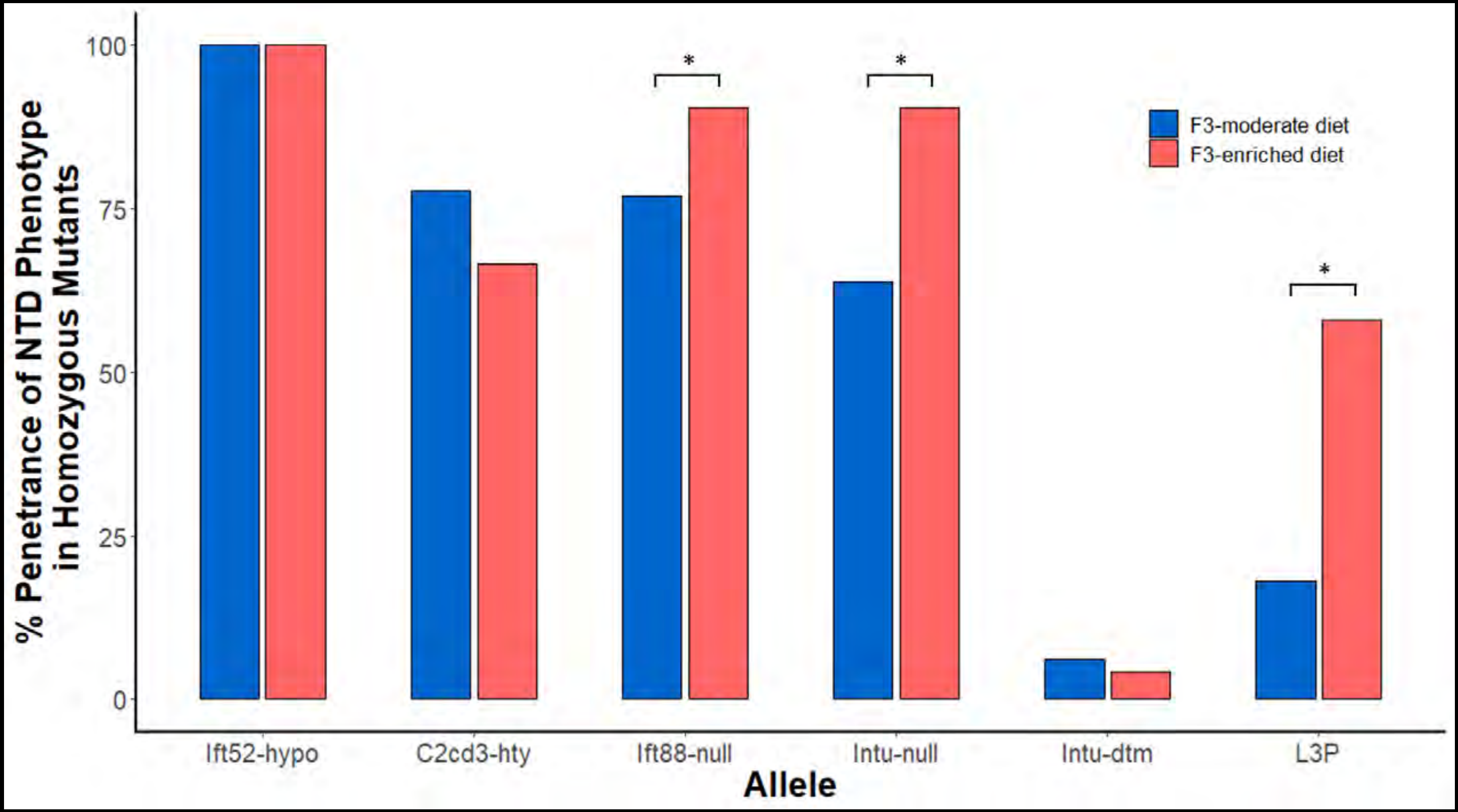




Ciliopathies



Folic Acid Response in Mouse Models

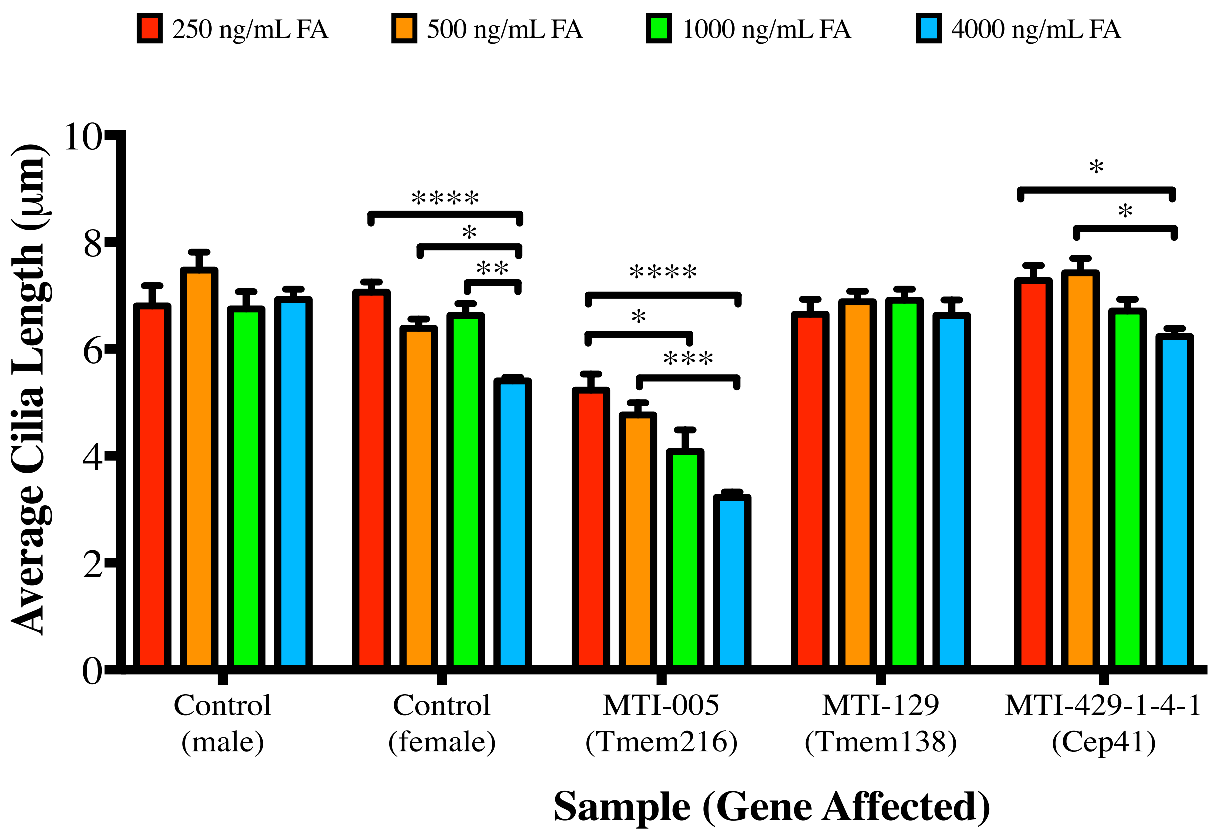


Non-responsive **Detrimental response**

Moderate folic acid levels are beneficial for cilia mutants

Moderate folic acid levels are beneficial for cilia mutants

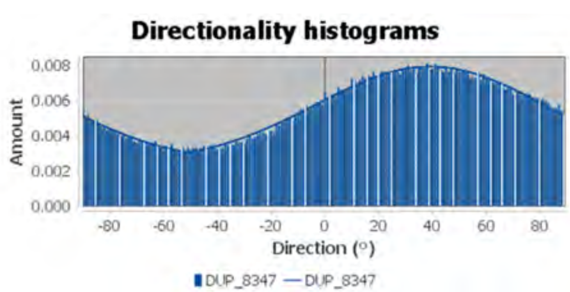
Human patient cell lines (primary cilia)



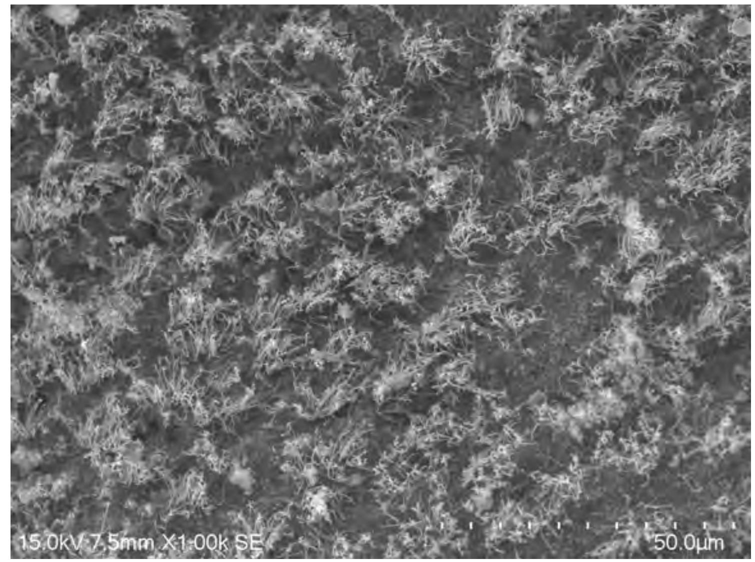
Moderate folic acid levels are beneficial for multi-ciliated cells

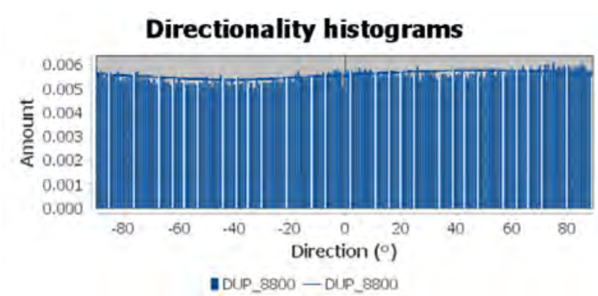
Ependymal flow (multi-ciliated cells in brain ventricles that move CSF)

Moderate folic acid diet



Enriched folic acid diet



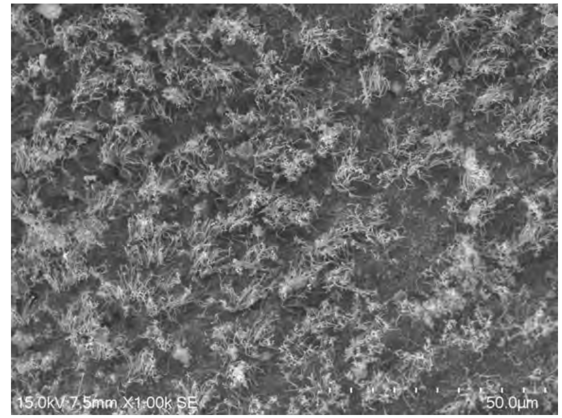


Moderate folic acid levels are beneficial for multi-ciliated cells

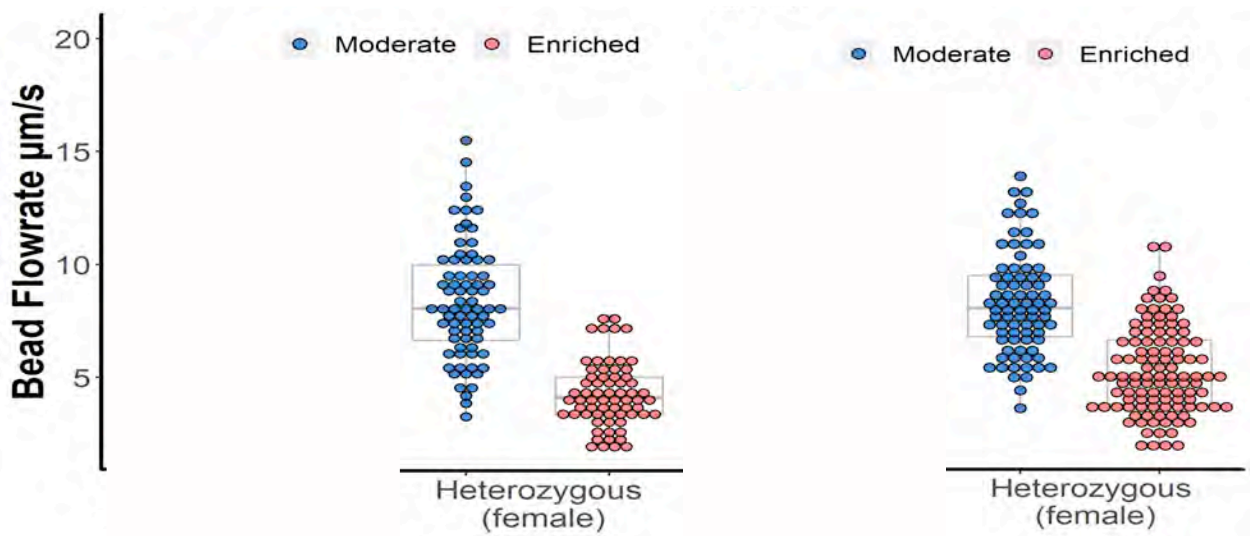
Ependymal flow (multi-ciliated cells in brain ventricles that move CSF)

Moderate folic acid diet

Enriched folic acid diet

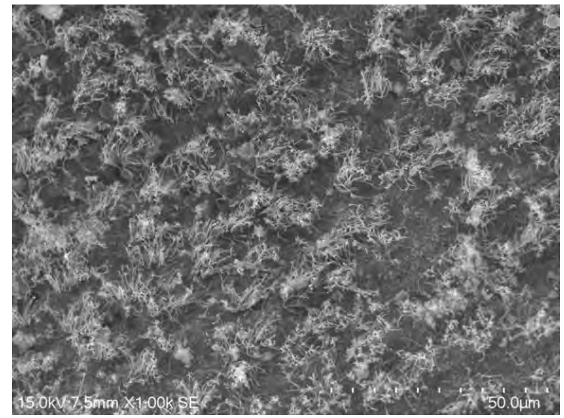


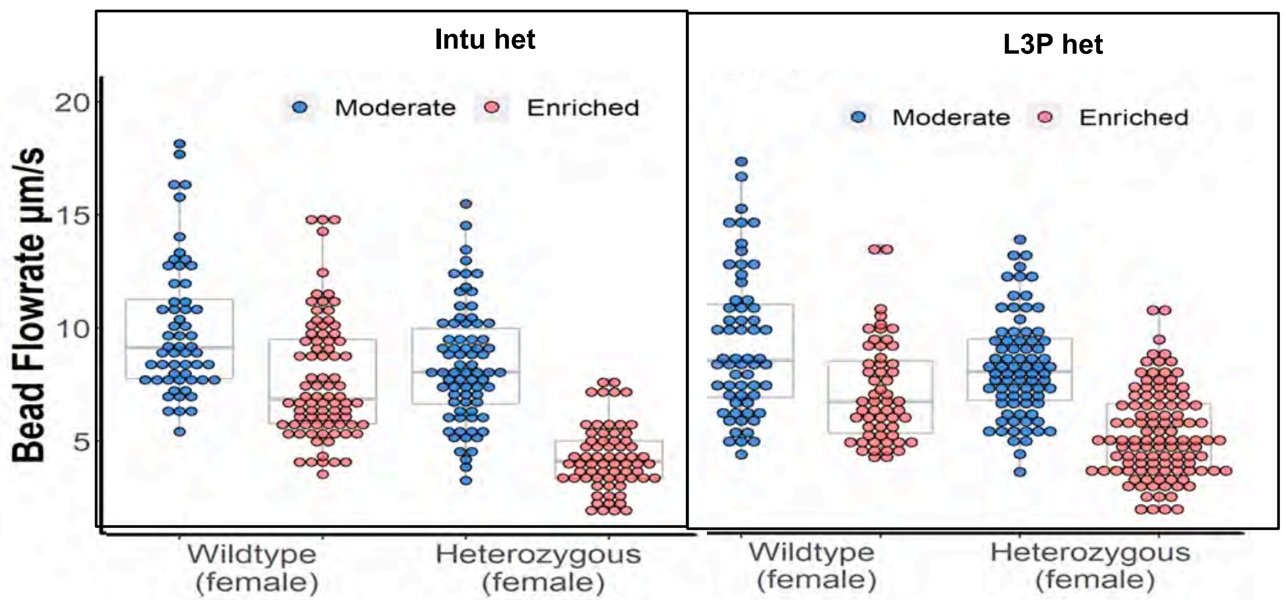
Intu het **L3P het**



Moderate folic acid levels are beneficial for multi-ciliated cells

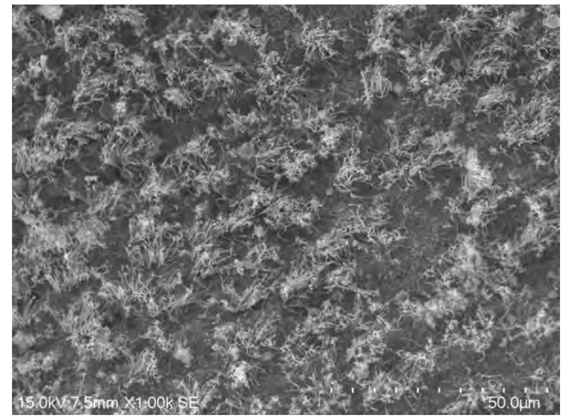
Ependymal flow (multi-ciliated cells in brain ventricles that move CSF)

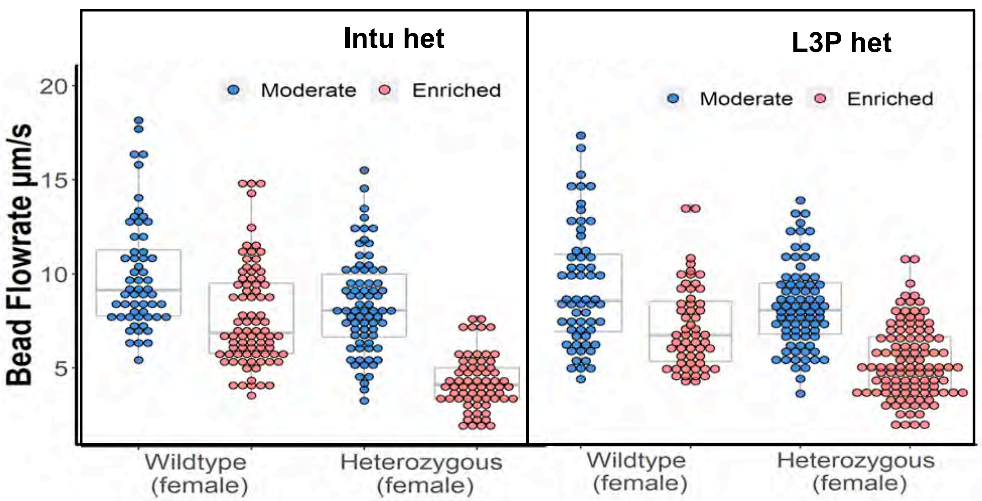


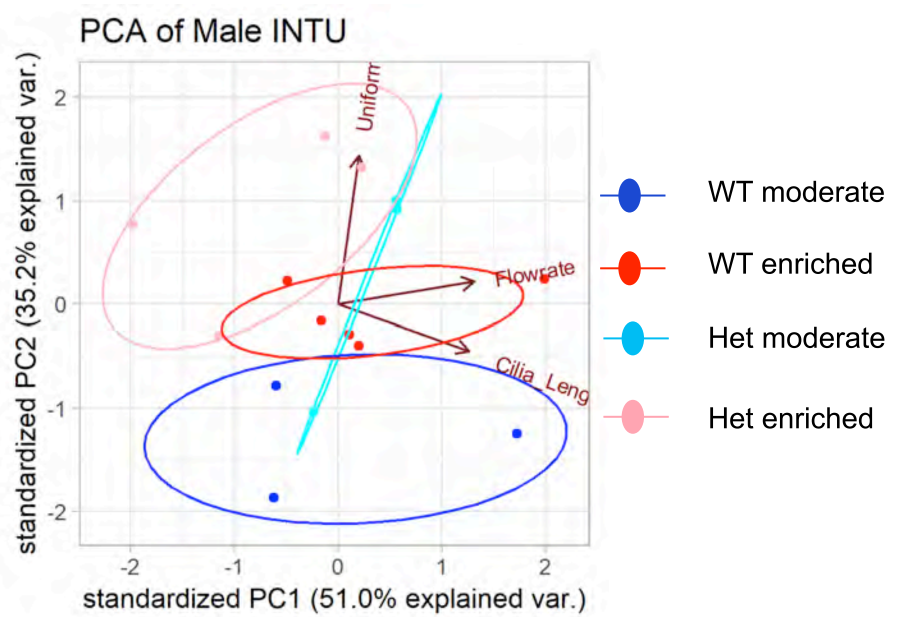


Moderate folic acid levels are beneficial for multi-ciliated cells

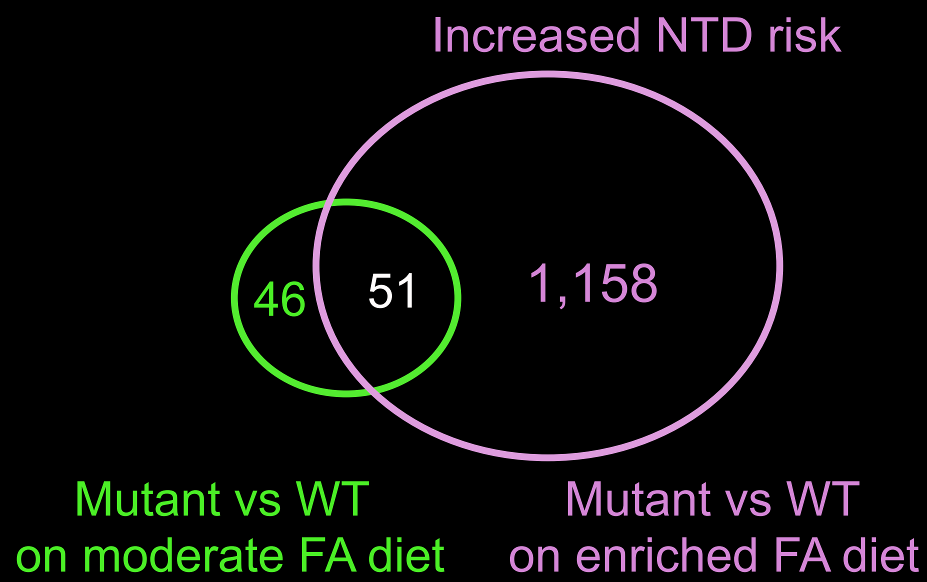
Ependymal flow (cilia in brain ventricles that move CSF)







Increased variability in gene expression as a contributor to NTD risk?



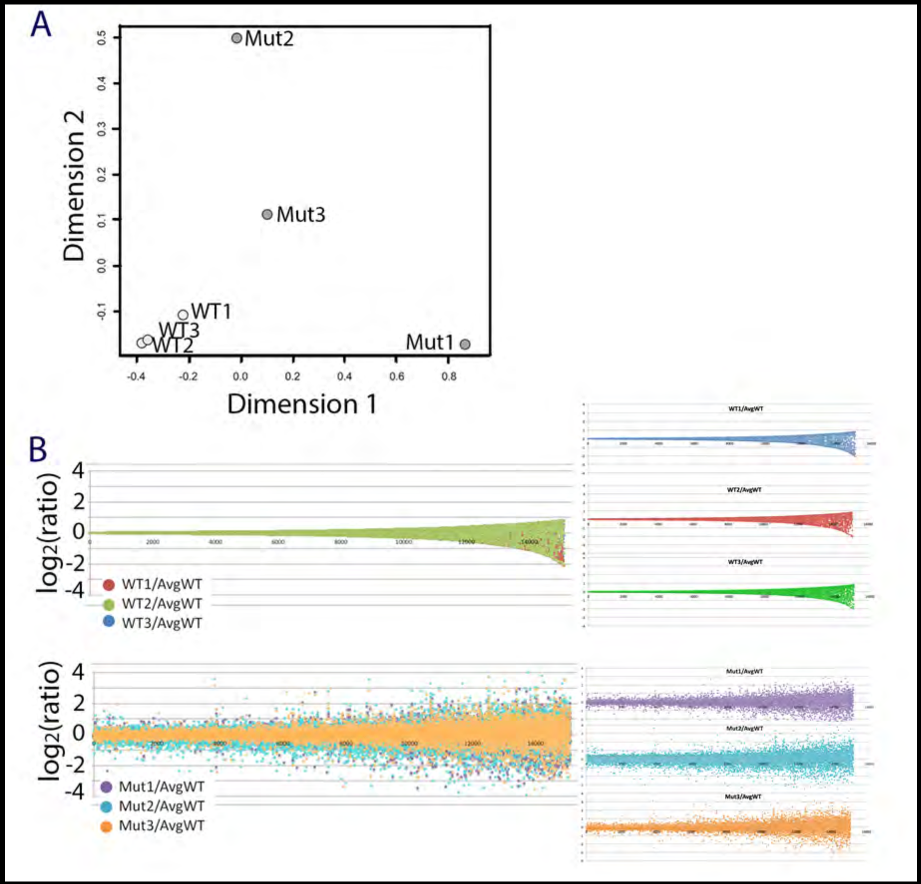
The genetics of an individual may determine the appropriate balance in folic acid supplementation

Inconsistent regulation of gene expression as a contributor to NTD risk

Baf155 mutant

ATP-dependent chromatin remodeling complex

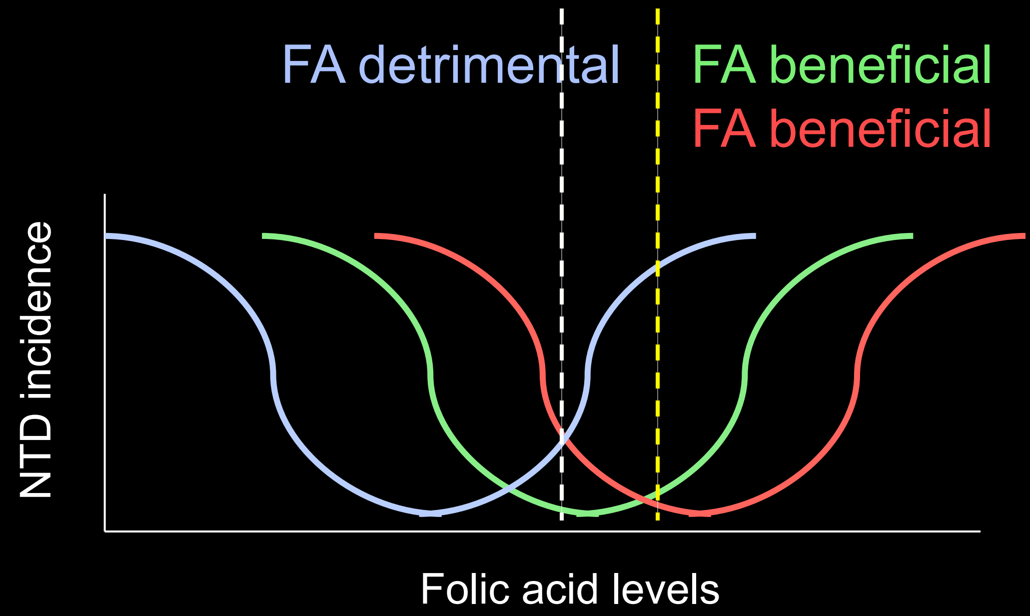
variable gene expression



Laura Harmacek
William Pavan (NIH)
Michael Salbaum
(Pennington Biomed Res, LA)

Deveiopmental Neurobiology 2013

Balance? Too MUCH, as well as too little may be problematic



Mutations can shift this balance

Is NTD prevention always due to rescue?

No, early embryonic lethality

Might some gene mutations and cellular processes benefit from moderate levels of folic acid?

Yes, cilia and others?

The genetics of an individual may determine the appropriate balance in folic acid supplementation

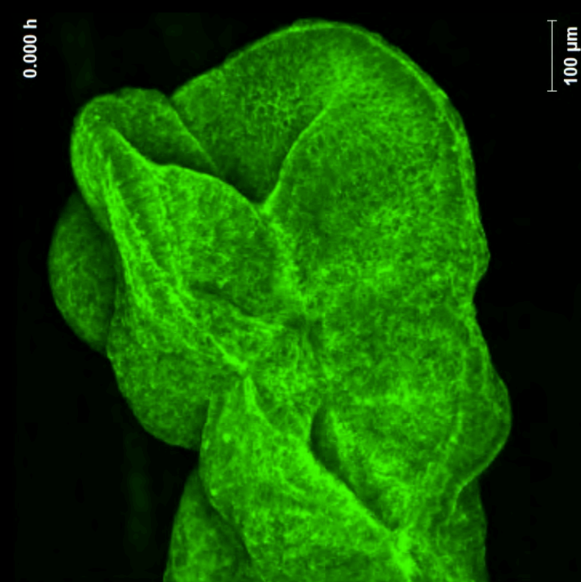
Approaches to Understand the Causes of NTDs

Genetics
Forward Genetic Screens
KOMP

Environment
Folic Acid
Zinc, Iron

Modeling Human NTD Mutations
Gleeson (UCSD)
Wang (Fudan Univ)
Zhang (Beijing)

Dynamic Imaging



Patient iPSCs
Maternal-Fetal Center
R. Marwan, MD

Lori Bulwith
Heather Clancy
David Engelhardt
Eric Jaffe

Binbin Li
Huili Li
Sofia Pezoa
Jing Zhang

Jianfu Chen (Univ of Southern California)
Amanda Graf (Nationwide Children's Hospital)
Laura Harmacek (National Jewish Health Center)
Tae-Hee Kim (Hospital for Sick Kids)
Aimin Liu (Penn State Univ)
Amber Marean (Univ of CO, Colorado Springs)
R'ada Massarwa (Weismann Institute)
Juliette Petersen (AAAS Fellow, State Dept)
Christina Pyrgaki (Rockefeller Univ Imaging Center)
Heather Ray (Univ of Alabama)
Carsten Schnatwinkel (Flagship Biosciences)
Jonathan Wilde (MIT)
Irene Zohn (Children's National Medical Center)
Ying Zhang (Harvard Univ)

Children's Hospital Colorado

HHMI HOWARD HUGHES MEDICAL INSTITUTE

NIH National Institute of Neurological Disorders and Stroke

SCHOOL OF MEDICINE RNA Bioscience Initiative UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Eunice Kennedy Shriver NICHD National Institute of Child Health & Human Development

<u>Collaborators</u>
Dr. K. Anderson
Dr. K. Hadjantonakis
Dr. P. Trainor
Dr. M. Justice
Dr. T. Zhang
Dr. H. Wang
Dr. J. Gleeson
Dr. R. Marwan
Dr. R. Bajpai